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(54) Title: CHIMERIC ADENOVIRAL VECTORS (57) Abstract A chimeric adenoviral vector is provided that comprises nucleotide sequence of a first adenovirus, wherein all or part of at least one gene of said first adenovirus encoding a protein that facilitates binding of said vector to a target mammalian cell, or internalization thereof within said cell, is replaced by all or part of the corresponding gene from a second adenovirus belonging to subgroup D, said vector further comprising a transgene operably linked to a eucaryotic promoter to allow for expression therefrom in a mammalian cell. Compositions comprising such vectors and methods of using such vectors to deliver transgenes to target mammalian cells, particularly airway epithelial cells, are also provided.		

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Description

Chimeric Adenoviral Vectors

5 Introduction

The present invention relates to chimeric adenoviral vectors, that is, vectors comprising DNA from more than one serotype of adenovirus, which offer enhanced infection efficiency of target cells in order to deliver one or more therapeutically useful nucleotide sequences, including transgenes, therein. Such a nucleotide
10 sequence may comprise a gene not otherwise present in the target cell that codes for a therapeutic and/or biologically active protein, or may represent, for example, an active copy of a gene that is already present in the target cell, but in a defective or deficient form.

15 Background of the Invention

One of the fundamental challenges now facing medical practitioners is that although the defective genes that are associated with numerous inherited diseases (or that represent disease risk factors including for various cancers) have been isolated and characterized, methods to correct the disease states themselves by providing
20 patients with normal copies of such genes (the technique of gene therapy) are substantially lacking. Accordingly, the development of improved methods of intracellular delivery therefor is of great medical importance. Examples of diseases that it is hoped can be treated by gene therapy include inherited disorders such as cystic fibrosis, Gaucher's disease, Fabry's disease, and muscular dystrophy.
25 Representative of acquired disorders that can be treated are: (1) for cancers: multiple myeloma, leukemias, melanomas, ovarian carcinoma and small cell lung cancer; (2) for cardiovascular conditions: progressive heart failure, restenosis, and hemophilias; and (3) for neurological conditions: traumatic brain injury.

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Gene therapy requires successful transfer of nucleic acid to the target cells of a patient. Gene transfer may generally be defined as the process of introducing an expressible polynucleotide (for example a gene, a cDNA, or an mRNA patterned thereon) into a cell. In a particular application of this approach, successful expression
5 of an encoding polynucleotide leads to production in the cells of a normal protein and leads to correction of a disease state associated with an abnormal gene. Therapies based on providing such proteins directly to target cells (protein replacement therapy) have generally proved ineffective since, for example, the cell membrane presents a selectively permeable barrier to entry. Thus there is great interest in alternative
10 methods to cause delivery of therapeutic proteins, especially by transfer of the relevant polynucleotide, often referred to as a transgene.

Viral vectors have been used with increasing frequency to date to deliver transgenes to target cells. Most attempts to use viral vectors for gene therapy have relied on retrovirus-based vectors, chiefly because of their ability to integrate into the
15 cellular genome. However, the disadvantages of retroviral vectors are becoming increasingly clear, including their tropism for dividing cells only, the possibility of insertional mutagenesis upon integration into the cell genome, decreased expression of the transgene over time, rapid inactivation by serum complement, and the possibility of generation of replication-competent retroviruses. See, for example, D. Jolly, et al.,
20 *Cancer Gene Therapy*, 1, 1994, pp. 51-64, and C.P. Hodgson, et al., *Bio Technology*, 13, 1995, pp. 222-225. Such disadvantages have led to the development of other viral-based vector systems, including those derived from adenoviruses.

Adenovirus (Ad) is a nuclear DNA virus with a genome of about 36 kb, which has been well-characterized through studies in classical genetics and molecular
25 biology. A detailed discussion of adenovirus is found in Thomas Shenk, "Adenoviridae and their Replication", and M. S. Horwitz, "Adenoviruses", Chapters 67 and 68, respectively, in *Virology*, B.N. Fields et al., eds., 2nd edition, Raven Press, Ltd., New York, 1996, and reference therein is found to numerous aspects of adenovirus pathology, epidemiology, structure, replication, genetics and classification.

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In a simplified form, the adenoviral genome is classified into early (known as E1-E4) and late (known as L1-L5) transcriptional units, referring to the generation of two temporal classes of viral proteins. The demarcation between these events is viral DNA replication.

5 The human adenoviruses are divided into numerous serotypes (approximately 47, numbered accordingly and classified into 6 subgroups: A, B, C, D, E and F), based upon properties including hemagglutination of red blood cells, oncogenicity, DNA base and protein amino acid compositions and homologies, and antigenic relationships. Additional background information concerning Ad serotype
10 classification, including that for subgroup D, can be found, for example, in F. Deryckere et al., *Journal of Virology*, 70, 1996, pp. 2832-2841; and A. Bailey et al., *Virology*, 205, 1994, pp. 438-452, and in other art-recognized references.

Adenoviruses are nonenveloped, regular icosahedrons (having 20 triangular surfaces and 12 vertices) that are about 65-80 nm in diameter. A protein called fiber
15 projects from each of these vertices. The fiber protein is itself generally composed of 3 identical polypeptide chains, although the length thereof varies between serotypes. The protein coat (capsid) is composed of 252 subunits (capsomeres), of which 240 are hexons, and 12 are pentons. Each penton comprises a penton base, on the surface of the capsid, and a fiber protein projecting from the base. The Ad 2 penton base protein,
20 for example, has been determined to be a 8 x 9 nm ring shaped complex composed of 5 identical protein subunits of 571 amino acids each.

Current understanding of adenovirus-cell interactions suggests that adenovirus utilizes two cellular receptors to attach to, and then infect a target cell. It has been further suggested that the fiber protein of an infecting adenovirus first attaches to a
25 receptor, the identity of which is still unknown, and then penton base attaches to a further receptor, often a protein of the alpha integrin family. It has been determined that alpha-integrins often recognize short amino acid sequences on other cellular proteins for attachment purposes including the tripeptide sequence Arg-Gly-Asp (abbreviated RGD). An RGD sequence is also found in the penton base protein of

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adenovirus and is currently understood in the art to mediate attachment of Ad to alpha integrins.

Recombinant adenoviruses have several advantages for use as gene transfer vectors, including tropism for both dividing and non-dividing cells, minimal
5 pathogenic potential, ability to replicate to high titer for preparation of vector stocks, and the potential to carry large inserts (Berkner, K.L., *Curr. Top. Micro. Immunol.* 158:39-66, 1992; Jolly, D., *Cancer Gene Therapy* 1:51-64, 1994).

The carrying capacity of an adenovirus vector is proportional to the size of the adenovirus genome present in the vector. For example, a capacity of about 8 kb can
10 be created from the deletion of certain regions of the virus genome dispensable for virus growth, e.g., E3, and the deletion of a genomic region such as E1 whose function may be restored in trans from 293 cells (Graham, F.L., *J. Gen. Virol.* 36:59-72, 1977) or A549 cells (Imler et al., *Gene Therapy* 3:75-84, 1996). Such E1-deleted vectors are rendered replication-defective, which is desirable for the engineering of adenoviruses
15 for gene transfer. The upper limit of vector DNA capacity for optimal carrying capacity is about 105%-108% of the length of the wild-type genome. Further adenovirus genomic modifications are possible in vector design using cell lines which supply other viral gene products in trans, e.g., complementation of E2a (Zhou et al., *J. Virol.* 70:7030-7038, 1996), complementation of E4 (Krougliak et al., *Hum. Gene*
20 *Ther.* 6:1575-1586, 1995; Wang et al., *Gene Ther.* 2:775-783, 1995), or complementation of protein IX (Caravokyri et al., *J. Virol.* 69:6627-6633, 1995; Krougliak et al., *Hum. Gene Ther.* 6:1575-1586, 1995). Maximal carrying capacity can be achieved using adenoviral vectors deleted for all viral coding sequences (Kochanek et al., *Proc. Natl. Acad. Sci. USA* 93:5731-5736, 1996; Fisher et al.,
25 *Virology* 217:11-22, 1996).

Transgenes that have been expressed to date by adenoviral vectors include p53 (Wills et al., *Human Gene Therapy* 5:1079-188, 1994); dystrophin (Vincent et al., *Nature Genetics* 5:130-134, 1993; erythropoietin (Descamps et al., *Human Gene Therapy* 5:979-985, 1994; ornithine transcarbamylase (Stratford-Perricaudet et al.,

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Human Gene Therapy 1:241-256, 1990; We et al., J. Biol. Chem. 271:3639-3646, 1996;); adenosine deaminase (Mitani et al., Human Gene Therapy 5:941-948, 1994); interleukin-2 (Haddada et al., Human Gene Therapy 4:703-711, 1993); and α 1-antitrypsin (Jaffe et al., Nature Genetics 1:372-378, 1992); thrombopoietin
5 (Ohwada et al., Blood 88:778-784, 1996); and cytosine deaminase (Ohwada et al., Hum. Gene Ther. 7:1567-1576, 1996).

The particular tropism of adenoviruses for cells of the respiratory tract has particular relevance to the use of adenovirus in gene therapy for cystic fibrosis (CF), which is the most common autosomal recessive disease in Caucasians. The disease is
10 caused by the presence of one or more mutations in the gene that encodes a protein known as cystic fibrosis transmembrane conductance regulator (CFTR), and which regulates the movement of ions (and therefore fluid) across the cell membrane of epithelial cells, including lung epithelial cells. Abnormal ion transport in airway cells leads to abnormal mucous secretion, inflammation and infection, tissue damage,
15 and eventually death. Mutations in the CFTR gene that disturb the cAMP-regulated Cl^- channel in airway epithelia result in pulmonary dysfunction (Zabner et al., Nature Genetics 6:75-83, 1994). Adenovirus vectors engineered to carry the CFTR gene have been developed (Rich et al., Human Gene Therapy 4:461-476, 1993) and studies have shown the ability of these vectors to deliver CFTR to nasal epithelia of CF patients
20 (Zabner et al., Cell 75:207-216, 1993), the airway epithelia of cotton rats and primates (Zabner et al., Nature Genetics 6:75-83, 1994), and the respiratory epithelium of CF patients (Crystal et al., Nature Genetics 8:42-51, 1994). Recent studies have shown that administering an adenoviral vector containing a DNA sequence encoding CFTR to airway epithelial cells of CF patients can restore a functioning chloride ion channel
25 in the treated epithelial cells (Zabner et al., J. Clin. Invest. 97:1504-1511, 1996; U.S. Patent No. 5,670,488 issued September 23, 1997).

Serotype classification is partly based on viral surface protein sequence variation. Because the infectious capabilities of the virus are associated with the surface protein interactions of the virus with cellular proteins, the serotype is an

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important determinant of viral entry into target cells, and can account for the infectious heterogeneity of adenovirus serotypes. Most adenoviral vectors have been constructed using adenovirus serotypes from the well-studied group C adenoviruses, especially Ad 2 and Ad 5. However, other adenovirus serotypes display infectious
5 properties that are relevant to the further design of improved adenoviral vectors, for example, those derived from subgroup D, which display enhanced tropism for human airway epithelial cells.

It is widely hoped that gene therapy will provide a long lasting and predictable form of therapy for certain disease states, and it is likely the only form of therapy
10 suitable for many inherited diseases. Although adenoviral vectors are currently in clinical use and have shown therapeutic promise, a need remains to improve the infection efficiency of these vectors in order to further improve their gene transfer capabilities. The present invention addresses this goal.

15 Summary Of The Invention

The present invention provides for chimeric adenoviral vectors which offer enhanced infection efficiency of target cells for the delivery of one or more transgenes. In a representative aspect of the invention, the vectors comprise nucleotide sequences coding for therapeutically useful proteins and have enhanced tropism for airway
20 epithelial cells.

Accordingly, there are provided chimeric adenoviral vectors comprising nucleotide sequence of a first adenovirus, wherein at least one gene of said first adenovirus encoding a protein that facilitates binding of said vector to a target mammalian cell, or internalization thereof within said cell, is replaced by the
25 corresponding gene from a second adenovirus belonging to subgroup D. These vectors may further comprising a transgene operably linked to a eucaryotic promoter or other regulatory elements to allow for expression therefrom in a mammalian cell. In a representative aspect thereof, the replaced encoding sequence codes for Ad fiber, hexon or penton base.

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In a further preferred embodiment of the invention, there are provided chimeric adenoviral vectors comprising nucleotide sequence of a first adenovirus, wherein a portion of a gene thereof encoding a protein that facilitates binding of said vector to a target mammalian cell, or internalization thereof within said cell, is replaced by a
5 portion of the corresponding gene from a second adenovirus belonging to subgroup D. These vectors may further comprising a transgene operably linked to a eucaryotic promoter or other regulatory elements to allow for expression therefrom in a mammalian cell. In a representative aspect thereof, the replaced encoding sequence codes for a portion of Ad fiber, hexon or penton base.

10 Preferably, the second adenovirus is a member of subgroup D, and the replaced nucleotide sequence encodes a polypeptide selected from the group consisting of Ad fiber, a fragment of Ad fiber, Ad hexon, a fragment of Ad hexon, Ad penton base, and a fragment of Ad penton base. In a preferred embodiment, said second adenovirus is selected from the group consisting of serotypes Ad 9, Ad 15, Ad
15 17, Ad 19, Ad 20, Ad 22, Ad 26, Ad 27, Ad 28, Ad 30, and Ad 39. In preferred embodiments of the chimeric adenoviral vectors, the first adenovirus is selected from the group consisting of Ad 2, Ad 5, and Ad 12.

The invention is also directed to compositions comprising the chimeric adenoviral vectors of the invention. Additional aspects of the invention include
20 methods to use the chimeric adenoviral vectors of the invention to deliver transgenes to mammalian target cells, for example, to the airway epithelial cells of patients.

A still further representative aspect of the invention involves a method of providing a therapeutic and/or biologically active protein to the airway epithelial cells of a patient by administering to said cells an adenoviral vector comprising elements of
25 an Ad 17 genome, and a transgene encoding said therapeutic protein that is operably linked to a eucaryotic promoter to allow for expression therefrom in a mammalian cell, under conditions whereby the transgene encoding said therapeutic protein is expressed, and therapeutic benefit is produced in said airway epithelial cells.

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These and other aspects of the present invention are described in the Detailed Description of the Invention which follows directly.

Brief Description of the Drawings

5 FIGURE 1 depicts infection of NHBE cells by Ad 2.

FIGURE 2 depicts infection of NHBE cells by Ad 17.

FIGURE 3 plots the result of binding to human nasal polyp epithelial cell isolates by Ad 2 and Ad 17.

FIGURE 4 is a map of the vector Ad2/ β gal-2/fiber Ad 17.

10 FIGURE 5 shows a comparison of the amino acid sequence of penton base from Ad 17 (top) [SEQ ID NO: 4] and Ad 2 (bottom) [SEQ ID NO: 5], and further depicts the variable RGD containing region.

FIGURE 6 depicts an amino acid sequence pileup for penton base from particular Ad serotypes, including fl0 (from fowl) [SEQ ID NO: 6 through SEQ ID
15 NO: 10].

FIGURE 7 shows a comparison of the amino acid sequence of fiber from Ad 17 (top) [SEQ ID NO: 11] and Ad 2 (bottom) [SEQ ID NO: 12].

FIGURE 8 depicts an amino acid sequence pileup for fiber from particular Ad serotypes [SEQ ID NO: 11 through SEQ ID NO: 22], including two forms of serotype
20 40 (40-1 and 40-2) which differ in that one variant has two (but non-identical) copies of the fiber gene.

FIGURE 9 shows the infection efficiency of colon cancer cell lines by adenovirus serotypes.

FIGURE 10 shows the infection efficiency of cancer cell lines by adenovirus
25 serotypes.

Provided in the Sequence Listing attached hereto are also:

SEQ ID NO: 1, the complete nucleotide sequence of Ad 17;

SEQ ID NO: 2, the complete encoding nucleotide sequence for Ad 17 fiber;

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SEQ ID NO: 3, the complete encoding nucleotide sequence for Ad 17 penton base.

Detailed Description of the Invention

5 The present invention provides for chimeric adenoviral vectors comprising nucleotide sequence of a first adenovirus, wherein at least one gene of said first adenovirus encoding a protein that facilitates binding of said vector to a target mammalian cell, or internalization thereof within said cell, is replaced by the corresponding gene from a second adenovirus belonging to subgroup D, said vectors
10 further comprising a transgene operably linked to a eucaryotic promoter to allow for expression therefrom in a mammalian cell. In a representative aspect thereof, the replaced encoding sequence correspond to the gene encoding the Ad fiber, hexon or penton base proteins, or combinations thereof.

 In a further preferred embodiment of the invention, there are provided chimeric
15 adenoviral vectors comprising nucleotide sequence of a first adenovirus, wherein a portion of a gene thereof encoding a protein that facilitates binding of said vector to a target mammalian cell, or internalization thereof within said cell, is replaced by a portion of the corresponding gene from a second adenovirus belonging to subgroup D, said vectors further comprising a transgene operably linked to a eucaryotic promoter to
20 allow for expression therefrom in a mammalian cell. In a representative aspect thereof, the replaced encoding sequence codes for a portion of the Ad fiber, hexon or penton base proteins, or combinations thereof. Where a portion of a gene from a second adenovirus is used to construct a chimeric adenoviral vector, such sequence will have a length sufficient to confer a desired serotypic-specific virus-cell interaction to the
25 vector.

 The present invention involves the recognition that adenoviral vectors that are either based substantially upon the genome of Ad serotypes classified in subgroup D, or that contain certain Ad-protein encoding polynucleotide sequences of subgroup D adenovirus, are particularly effective at binding to, and internalizing within, human

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cells, such that therapeutic transgenes included in the adenoviral vector are efficiently expressed. This discovery is particularly surprising given that adenovirus serotypes of subgroup D are not clinically associated with human respiratory disease, and that, for example association with conjunctivitis is more typical. The recognition of this

5 tropism is of particular relevance for the treatment by gene therapy of recognized disease states such as cystic fibrosis or α 1-antitrypsin deficiency. This discovery is particularly surprising given that adenovirus serotypes of subgroup D are not clinically associated with human respiratory disease, and that, for example association with

10 conjunctivitis is more typical. The recognition of this tropism is of particular relevance for the treatment by gene therapy of recognized disease states such as cystic fibrosis or α 1-antitrypsin deficiency.

In a representative aspect of the invention, the adenoviral vectors further comprise nucleotide sequences coding for one or more transgenes and have enhanced tropism for airway epithelial cells. Preferably, the chimeric adenoviral vectors are

15 replication-defective, a feature which contributes to the enhanced safety of adenoviral vectors administered to individuals.

Preferably, the second adenovirus is a member of subgroup D, and the replaced nucleotide sequence encodes a polypeptide selected from the group consisting of Ad fiber, a fragment of Ad fiber, Ad hexon, a fragment of Ad hexon, Ad penton base, and

20 a fragment of Ad penton base. In a preferred embodiment, said second adenovirus is selected from the group consisting of serotypes Ad 9, Ad 15, Ad 17, Ad 19, Ad 20, Ad 22, Ad 26, Ad 27, Ad 28, Ad 30, and Ad 39. In a most preferred embodiment, the second adenovirus is Ad 17. In other preferred embodiments of the chimeric adenoviral vectors, the first adenovirus is selected from the group consisting of Ad 2,

25 Ad 5, and Ad 12.

There is substantial evidence that any reported transforming properties of the E4 region of certain subgroup D serotypes do not extend to Ad serotypes whose use is preferred according to the practice of the present invention (see, for example, R. Javier

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et al., Science, 257, 1992, pp. 1267-1271). It is expected also that, for example, individual ORFs of subgroup D E4 region, such as ORF1, could be deleted.

Additional aspects of the invention include methods to provide biologically active and/or therapeutic proteins to mammalian cells, including, but not limited to, the airway epithelial cells of individuals, in order to provide phenotypic benefit. According to this aspect of the invention, chimeric adenoviral vectors are used in which a nucleotide sequence of a first adenovirus is replaced by the corresponding nucleotide sequence of a second adenovirus. Preferably, the second adenovirus is a member of subgroup D, and the replaced nucleotide sequence encodes a polypeptide encoding all or part of Ad fiber, Ad hexon, or Ad penton base, or combinations thereof.

A still further representative aspect of the invention involves providing a biologically active and/or therapeutic protein in the airway epithelial cells of a patient by administering to said cells an adenoviral vector comprising elements of an Ad 17 genome, and a transgene encoding said protein that is operably linked to a eucaryotic promoter to allow for expression therefrom in a mammalian cell, under conditions whereby the transgene encoding said protein is expressed, and the desired phenotypic benefit is produced in said airway epithelial cells. According to the practice of the invention, it is preferred that an chimeric adenovirus vector utilized to deliver a transgene to the respiratory epithelium (including that of the nasal airway, trachea, and bronchi and alveoli of the lung), or to other tissues of the body, comprise serotypes within subgroup D, as such classification is recognized in the art.

In order to construct the chimeric adenoviral vectors of the invention, reference may be made to the substantial body of literature on how such vectors may be designed, constructed and propagated using techniques from molecular biology and microbiology that are well-known to the skilled artisan. Specific examples of adenoviral vector genomes which can be used as the backbone for a chimeric adenoviral vector of the invention include, for example, Ad2/CFTR-1 and Ad2/CFTR-2 and others described in U. S. Patent No. 5,670,488, issued September 23, 1997

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(incorporated herein by reference). Such vectors may include deletion of the E1 region, partial or complete deletion of the E4 region, and deletions within, for example, the E2 and E3 regions. Within the scope of the invention are, for example, chimeric vectors which contain an Ad 2 backbone with one or more Ad 17 capsid proteins or fragments thereof in the virus. Other adenoviral vector genomic designs which can be used in the chimeric adenoviral vectors of the invention include those derived from allowed U.S. Patent Application Serial No. 08/409,874, filed March 24, 1995, and allowed U.S. Patent Application Serial No. 08/540,077, filed October 6, 1995 (both incorporated herein by reference).

10 To construct the recombinant chimeric adenoviral vectors of the invention which contain a transcription unit, the skilled artisan can use the standard techniques of molecular biology to engineer a transgene or a capsid protein into a backbone vector genome (Berkner, K.L., Curr. Top. Micro. Immunol. 158:39-66, 1992). For example, a plasmid containing a transgene and any operably linked regulatory elements inserted into an adenovirus genomic fragment can be co-transfected with a linearized viral genome derived from an adenoviral vector of interest into a recipient cell under conditions whereby homologous recombination occurs between the genomic fragment and the virus. Preferably, a transgene is engineered into the site of an E1 deletion. As a result, the transgene is inserted into the adenoviral genome at the site in which it was cloned into the plasmid, creating a recombinant adenoviral vector. The chimeric adenoviral vectors can also be constructed using standard ligation techniques, for example, removing a restriction fragment containing a fiber gene from a first adenovirus and ligating into that site a restriction fragment containing a fiber gene from a second adenovirus. A representative example of a chimeric adenoviral vector of the invention is Ad2/ β gal-2 fiber 17 (exemplified in Example 6).

25 Construction of the chimeric adenoviral vectors can be based on adenovirus DNA sequence information widely available in the field, e.g., nucleic acid sequence databases such as GenBank.

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Preparation of replication-defective chimeric adenoviral vector stocks can be accomplished using cell lines that complement viral genes deleted from the vector, e.g., 293 or A549 cells containing the deleted adenovirus E1 genomic sequences. The use of HER3 cells (human embryonic retinoblasts transformed by Ad 12), as a
5 complementing cell line is of note. After amplification of plaques in suitable complementing cell lines, the viruses can be recovered by freeze-thawing and subsequently purified using cesium chloride centrifugation. Alternatively, virus purification can be performed using chromatographic techniques, e.g., as set forth in International Application No. PCT/US96/13872, filed August 30, 1996, incorporated
10 herein by reference.

Titers of replication-defective chimeric adenoviral vector stocks can be determined by plaque formation in a complementing cell line, e.g., 293 cells. End-point dilution using an antibody to the adenoviral hexon protein may be used to quantitate virus production or infection efficiency of target cells (Armentano et al.,
15 Hum. Gene Ther. 6:1343-1353, 1995, incorporated herein by reference).

Transgenes which can be delivered and expressed from a chimeric adenoviral vector of the invention include, but are not limited to, those encoding enzymes, blood derivatives, hormones, lymphokines such as the interleukins and interferons, coagulants, growth factors, neurotransmitters, tumor suppressors, apolipoproteins,
20 antigens, and antibodies, and other biologically active proteins. Specific transgenes which may be encoded by the chimeric adenoviral vectors of the invention include, but are not limited to, cystic fibrosis transmembrane regulator (CFTR), dystrophin, glucocerebrosidase, tumor necrosis factor, p53, p21, herpes simplex thymidine kinase and gancyclovir, retinoblastoma (Rb), and adenosine deaminase (ADA). Transgenes
25 encoding antisense molecules or ribozymes are also within the scope of the invention. The vectors may contain one or more transgenes under the control of one or more regulatory elements.

In addition to containing the DNA sequences encoding one or more transgenes, the chimeric adenoviral vectors of the invention may contain any

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expression control sequences such as a promoter or enhancer, a polyadenylation element, and any other regulatory elements that may be used to modulate or increase expression, all of which are operably linked in order to allow expression of the transgene. The use of any expression control sequences, or regulatory elements,
5 which facilitate expression of the transgene is within the scope of the invention. Such sequences or elements may be capable of generating tissue-specific expression or be susceptible to induction by exogenous agents or stimuli.

Infection of target cell by the chimeric adenoviral vectors of the invention may also be facilitated by the use of cationic molecules, such as cationic lipids as disclosed
10 in PCT Publication No. WO96/18372, published June 20, 1996, incorporated herein by reference.

Cationic amphiphiles have a chemical structure which encompasses both polar and non-polar domains so that the molecule can simultaneously facilitate entry across a lipid membrane with its non-polar domain while its cationic polar domain attaches
15 to a biologically useful molecule to be transported across the membrane.

Cationic amphiphiles which may be used to form complexes with the chimeric adenoviral vectors of the invention include, but are not limited to, cationic lipids, such as DOTMA (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, 1987) (N-[1-(2,3-dioletoxy)propyl]-N,N,N - trimethylammonium chloride); DOGS
20 (dioctadecylamidoglycylspermine) (Behr et al., Proc. Natl. Acad. Sci. USA 86:6982-6986, 1989); DMRIE (1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide) (Felgner et al., J. Biol. Chem. 269:2550-2561, 1994; and DC-chol (3B [N-N', N'-dimethylaminoethane) -carbamoyl] cholesterol) (U.S. Patent No. 5, 283,185 to Eband et al.). The use of other cationic amphiphiles recognized in the art or which
25 come to be discovered is within the scope of the invention.

In preferred embodiments of the invention, the cationic amphiphiles useful to complex with and facilitate transfer of the vectors of the invention are those lipids which are described in PCT Publication No. WO96/18372, published June 20, 1996, which is incorporated herein by reference. Preferred cationic amphiphiles described

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herein to be used in the delivery of the plasmids and/or viruses are GL-53, GL-67, GL-75, GL-87, GL-89, and GL-120, including protonated, partially protonated, and deprotonated forms thereof. Further embodiments include the use of non-T-shaped amphiphiles as described on pp. 22-23 of the aforementioned PCT application, 5 including protonated, partially protonated and deprotonated forms thereof. Most preferably, the cationic amphiphile which can be used to deliver the vectors of the invention is spermine cholesterol carbamate (GL-67).

In the formulation of compositions comprising the chimeric adenoviral vectors of the invention, one or more cationic amphiphiles may be formulated with neutral co- 10 lipids such as dileoylphosphatidylethanolamine (DOPE) to facilitate delivery of the vectors into a cell. Other co-lipids which may be used in these complexes include, but are not limited to, diphytanoylphosphatidylethanolamine, lyso-phosphatidylethanolamines, other phosphatidylethanolamines, phosphatidylcholines, lyso-phosphatidylcholines and cholesterol. A preferred molar ratio of cationic 15 amphiphile to colipid is 1:1. However, it is within the scope of the invention to vary this ratio, including also over a considerable range. In a preferred embodiment of the invention, the cationic amphiphile GL-67 and the neutral co-lipid DOPE are combined in a 1:2 molar ratio, respectively, before complexing with a chimeric adenoviral vector for delivery to a cell.

20 In the formulation of complexes containing a cationic amphiphile with a chimeric adenoviral vector, a preferred range of 10^7 - 10^{10} infectious units of virus may be combined with a range of 10^4 - 10^6 cationic amphiphile molecules/viral particle.

The infection efficiency of the chimeric adenoviral vectors of the invention 25 may be assayed by standard techniques to determine the infection of target cells. Such methods include, but are not limited to, plaque formation, end-point dilution using, for example, an antibody to the adenoviral hexon protein, and cell binding assays using radiolabelled virus. Improved infection efficiency may be characterized as an increase in infection of at least an order of magnitude with reference to a control virus. Where

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a chimeric adenoviral vector encodes a marker or other transgene, relevant molecular assays to determine expression include the measurement of transgene mRNA, by, for example, Northern blot, S1 analysis or reverse transcription-polymerase chain reaction (RT-PCR). The presence of a protein encoded by a transgene may be detected by

5 Western blot, immunoprecipitation, immunocytochemistry, or other techniques known to those skilled in the art. Marker-specific assays can also be used, such as X-gal staining of cells infected with a chimeric adenoviral vector encoding β -galactosidase.

In order to determine transgene expression and infection efficiency in vivo using the constructs and compositions of the invention, animal models may be

10 particularly relevant in order to assess transgene persistence against a background of potential host immune response. Such a model may be chosen with reference to such parameters as ease of delivery, identity of transgene, relevant molecular assays, and assessment of clinical status. Where the transgene encodes a protein whose lack is associated with a particular disease state, an animal model which is representative of

15 the disease state may optimally be used in order to assess a specific phenotypic result and clinical improvement. However, it is also possible that particular chimeric adenoviral vectors of the invention display enhanced infection efficiency only in human model systems, e.g., using primary cell cultures, tissue explants, or permanent cell lines. In such circumstances where there is no animal model system available in

20 which to model the infection efficiency of a chimeric adenoviral vector with respect to human cells, reference to art-recognized human cell culture models will be most relevant and definitive.

Relevant animals in which the chimeric adenoviral vectors may be assayed include, but are not limited to, mice, rats, monkeys, and rabbits. Suitable mouse

25 strains in which the vectors may be tested include, but are not limited to, C3H, C57Bl/6 (wild-type and nude) and Balb/c (available from Taconic Farms, Germantown, New York).

Where it is desirable to assess the host immune response to vector administration, testing in immune-competent and immune-deficient animals may be

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compared in order to define specific adverse responses generated by the immune system. The use of immune-deficient animals, e.g., nude mice, may be used to characterize vector performance and persistence of transgene expression, independent of an acquired host response.

5 In a particular embodiment where the transgene is the gene encoding cystic fibrosis transmembrane regulator protein (CFTR) which is administered to the respiratory epithelium of test animals, expression of CFTR may be assayed in the lungs of relevant animal models, for example, C57Bl/6 or Balb/c mice, cotton rats, or Rhesus monkeys. Molecular markers which may be used to determine expression
10 include the measurement of CFTR mRNA, by, for example, Northern blot, S1 analysis or RT-PCR. The presence of the CFTR protein may be detected by Western blot, immunoprecipitation, immunocytochemistry, or other techniques known to those skilled in the art. Such assays may also be used in tissue culture where cells deficient in a functional CFTR protein and into which the chimeric adenoviral vectors have
15 been introduced may be assessed to determine the presence of functional chloride ion channels - indicative of the presence of a functional CFTR molecule.

The chimeric adenoviral vectors of the invention have a number of in vivo and in vitro utilities. The vectors can be used to transfer a normal copy of a transgene encoding a biologically active protein to target cells in order to remedy a deficient or
20 dysfunctional protein. The vectors can be used to transfer marked transgenes (e.g., containing nucleotide alterations) which allow for distinguishing expression levels of a transduced gene from the levels of an endogenous gene. The chimeric adenoviral vectors can also be used to define the mechanism of specific viral protein-cellular protein interactions that are mediated by specific virus surface protein sequences. The
25 vectors can also be used to optimize infection efficiency of specific target cells by adenoviral vectors, for example, using a chimeric adenoviral vector containing Ad 17 fiber protein to infect human nasal polyp cells. Where it is desirable to use an adenoviral vector for gene transfer to cancer cells in an individual, a chimeric adenoviral vector can be chosen which selectively infects the specific type of target

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cancer cell and avoids promiscuous infection. Where primary cells are isolated from a tumor in an individual requiring gene transfer, the cells may be tested against a panel of chimeric adenoviral vectors to select a vector with optimal infection efficiency for gene delivery. The vectors can further be used to transfer tumor antigens to dendritic
5 cells which can then be delivered to an individual to elicit an anti-tumor immune response. Chimeric adenoviral vectors can also be used to evade undesirable immune responses to particular adenovirus serotypes which compromise the gene transfer capability of adenoviral vectors.

The present invention is further directed to compositions containing the
10 chimeric adenoviral vectors of the invention which can be administered in an amount effective to deliver one or more desired transgenes to the cells of an individual in need of such molecules and cause expression of a transgene encoding a biologically active protein to achieve a specific phenotypic result. The cationic amphiphile-plasmid complexes or cationic amphiphile-virus complexes may be formulated into
15 compositions for administration to an individual in need of the delivery of the transgenes.

The compositions can include physiologically acceptable carriers, including any relevant solvents. As used herein, "physiologically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents,
20 isotonic and absorption delaying agents, and the like. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the compositions is contemplated.

Routes of administration for the compositions containing the chimeric adenoviral vectors of the invention include conventional and physiologically
25 acceptable routes such as direct delivery to a target organ or tissue, intranasal, intravenous, intramuscular, subcutaneous, intradermal, oral and other parenteral routes of administration.

The invention is further directed to methods for using the compositions of the invention in vivo or ex vivo applications in which it is desirable to deliver one or more

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transgenes into cells such that the transgene produces a biologically active protein for a normal biological or phenotypic effect. In vivo applications involve the direct administration of one or more chimeric adenoviral vectors formulated into a composition to the cells of an individual. Ex vivo applications involve the transfer of
5 a composition containing the chimeric adenoviral vectors directly to autologous cells which are maintained in vitro, followed by readministration of the transduced cells to a recipient.

Dosage of the chimeric adenoviral vector to be administered to an individual for expression of a transgene encoding a biologically active protein and to achieve a
10 specific phenotypic result is determined with reference to various parameters, including the condition to be treated, the age, weight and clinical status of the individual, and the particular molecular defect requiring the provision of a biologically active protein. The dosage is preferably chosen so that administration causes a specific phenotypic result, as measured by molecular assays or clinical markers. For
15 example, determination of the infection efficiency of a chimeric adenoviral vector containing the CFTR transgene which is administered to an individual can be performed by molecular assays including the measurement of CFTR mRNA, by, for example, Northern blot, S1 or RT-PCR analysis or the measurement of the CFTR protein as detected by Western blot, immunoprecipitation, immunocytochemistry, or
20 other techniques known to those skilled in the art. Relevant clinical studies which could be used to assess phenotypic results from delivery of the CFTR transgene include PFT assessment of lung function and radiological evaluation of the lung. Demonstration of the delivery of a transgene encoding CFTR can also be demonstrated by detecting the presence of a functional chloride channel in cells of an
25 individual with cystic fibrosis to whom the vector containing the transgene has been administered (Zabner et al., J. Clin. Invest. 97:1504-1511, 1996). Transgene expression in other disease states can be assayed analogously, using the specific clinical parameters most relevant to the condition.

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Dosages of a chimeric adenoviral vector which are effective to provide expression of a transgene encoding a biologically active protein and achieve a specific phenotypic result range from approximately 10^8 infectious units (I.U.) to 10^{11} I.U. for humans.

5 It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated, each unit containing a predetermined quantity of active ingredient calculated to produce the specific phenotypic effect in association with the required
10 physiologically acceptable carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly depend on the unique characteristics of the chimeric adenoviral vector and the limitations inherent in the art of compounding. The principal active ingredient (the chimeric adenoviral vector) is compounded for convenient and effective administration in effective amounts with the physiologically
15 acceptable carrier in dosage unit form as discussed above.

 Maximum benefit and achievement of a specific phenotypic result from administration of the chimeric adenoviral vectors of the invention may require repeated administration. Such repeated administration may involve the use of the same chimeric adenoviral vector, or, alternatively, may involve the use of different
20 chimeric adenoviral vectors which are rotated in order to alter viral antigen expression and decrease host immune response.

 The practice of the invention employs, unless otherwise indicated, conventional techniques of protein chemistry, molecular virology, microbiology, recombinant DNA technology, and pharmacology, which are within the skill of the
25 art. Such techniques are explained fully in the literature. See, e.g., Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, Inc., New York, 1995, and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, PA, 1985.

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The invention is further illustrated by the following specific examples which are not intended in any way to limit the scope of the invention.

Examples

5

Example 1 Infection of NHBE cells by adenovirus serotypes of subgroup D

Normal human bronchial epithelial ("NHBE") cells were obtained from Clonetics (San Diego, CA), and plated on Costar (Cambridge, MA) Transwell-Clear polyester membranes that were pre-coated with human placental collagen. The wells
10 were placed in a cluster plate and cells were fed every day for one week by changing the medium in both the well and the plate. After one week the media was removed from the wells to create an air-liquid interface, and the cells were then fed only by changing the medium in the cluster plate, every other day for one week. Cells were
15 infected at an moi of 1 by adding virus (see below) to the transwell, followed by an incubation time of 1.5-2 hours. At the end of the incubation period, the medium was removed and the cells were gently rinsed with fresh medium. Thirty-six hours post-infection the cells were fixed with 1:1 acetone:methanol, permeablized with a solution of 0.05% Tween 20 in PBS, and stained with FITC labeled anti-hexon antibody
(Chemicon, Temecula, CA) to visualize cells that had been productively infected (i.e.
20 to visualize virus replication). Cells were also subjected to the DAPI staining procedure in order to visualize the total number of nuclei. The results could be readily determined upon simple inspection.

Wild type Ad serotypes within subgroup D that were tested included 9, 15, 17, 19, 20, 22, 26, 27, 28, 30, and 39 (all from the American Type Culture Collection,
25 Rockville, MD). An Ad 2 (obtained as DNA from BRL, Gaithersburg, MD, and used to transfect 293 cells in order to generate virus stock) was used as a control. Infection observed with all of the subgroup D serotypes was superior to that observed with Ad 2, with the best results being achieved with Ad 9, Ad 17, Ad 20, Ad 22, and Ad 30.

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Additionally, it was determined that each of the above-mentioned serotypes of subgroup D was more effective in the NHBE cell assay under similar circumstances than any other serotype tested than belongs to a subgroup other than D. In this regard, the following serotypes were also tested: 31(subgroup A); 3(subgroup B); 7(subgroup B); 7a(subgroup B); 14(subgroup B); 4(subgroup E); and 41(subgroup F). In a further experiment, serotype 35 (subgroup A) may have performed as well as the least effective members of subgroup D that were tested.

Example 2 Infection of clinical isolate bronchial epithelial cells

Following generally the procedures of Example 1, human bronchial epithelial cells recovered from healthy human volunteers were infected with either Ad 2 (as above, Ad 2 DNA was obtained from BRL, and this DNA was used to transfect 293 cells to generate virus) (Figure 1), or Ad 17 (from ATCC) (Figure 2), all at an moi of 50. Cells were left in contact with virus for 30 minutes, 3 hours, or 12 hours.

The increased tropism of Ad 17 for human bronchial epithelial cells, compared with Ad 2, is readily apparent upon inspection of Figures 1 and 2. In the Figures, the right hand columns (panels D, E, and F, stained in blue) show total numbers of cells present (from DAPI staining as above), whereas the left hand columns (panels A, B, and C, stained in green) quantify adenovirus hexon protein present in the infected cells (from FITC-labeled anti-hexon antibody, as above). Panels A and D result from 30 minute incubation times, panels B and E result from 3 hour incubation times, and panels C and F result from 12 hour incubation times. As measured by the technique employed, infection of airway epithelia by Ad 17 is at least 50 fold greater than by Ad 2 for the thirty minute incubation time.

Example 3 Binding of Ad 2 and Ad 17 to human nasal polyp cell isolates

293 cells, a complementing cell line developed by Graham et al. (see Gen. Virol. , 36, 1977, pp. 59-72), were infected with either wild type Ad 2 or wild type Ad 17. Five hours post-infection the media was removed and replaced with methionine

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free media containing S^{35} metabolic label (Amersham). After an additional six hours, fresh media was added and the labeling was allowed to proceed for a total of 18 hours, after which the S^{35} media was removed and replaced with fresh media. Thirty hours post-infection the cells were harvested and lysed and the labeled Ad 2 or Ad 17
5 viruses were purified by CsCl gradient centrifugation. The recovered viruses were then used in an assay to determine their relative binding efficiency on human nasal polyp cells.

In order to perform the assay, ciliated human airway epithelial cells were recovered from nasal polyps of healthy volunteers. The results from two such isolates,
10 NP-14 and NP-15, are reported here (see Figure 3). Radiolabeled virus was then incubated with the isolated cells in wells for specified times (5 or 30 minutes, see Figure 3). The cells were then rinsed and measured for radioactivity. Binding as reported in Figure 3 indicates the percent of input radioactivity that is cell associated. It was determined that for both cell isolate populations, using either 5 or 30 minute
15 incubations, cell associated radioactivity was 10-fold enhanced if Ad 17 rather than Ad 2 was used.

Example 4 Fiber competition

20 A549 cells (a human lung carcinoma line, obtained from the American Type Culture Collection as ATCC CCL-185) were plated at 3×10^4 cells per well in 96-well dishes. Since the number of receptor sites for adenovirus fiber on the cell surface has been estimated to be approximately 10^5 receptors per cell, the receptors in the plated cells were saturated, in this example, with $0.1 \mu\text{g}$ of purified full length Ad 2 fiber
25 protein (obtained from Paul Freimuth, Brookhaven National Laboratory, Upton, NY), which corresponds to approximately 100 molecules of fiber per receptor. Cells were incubated with Ad 2 fiber in PBS for two hours at 37°C .

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The cells were subsequently infected at an moi of 1 (using either Ad 2 provided as above, or wild type Ad 17) for one hour, after which the cells were rinsed, and fresh medium was added. Control cultures were incubated with PBS with no added protein for two hours and then subsequently infected as described above. Forty
5 hours post-infection the cells were fixed with 1:1 acetone:methanol, permeabilized with 0.05% Tween 20 in PBS and stained with FITC labeled anti- Ad 2 hexon antibody, as described in Example 1. As determined by this assay, the number of cells infected (stained) with Ad 2 was reduced by approximately 90% in cultures that were pre-incubated with Ad 2 fiber as compared to control cultures. However, no effect on
10 Ad 17 infection was observed by the pre-incubation of A549 cells with full length Ad 2 fiber.

Example 5 Use of Ad 2 fiber knob in a binding competition
experiment with Ad 2

15

Further competition experiments were performed with Ad 2 and Ad 17 fiber knobs that had been expressed and purified from E. coli. DNA sequences encoding both protein fragments were designed so that the fiber knobs expressed therefrom would contain histidine tags in order to permit nickel- column purification. The yield
20 of soluble fiber knob trimer, purified by the Ni-NTA method (Qiagen, Chatsworth, CA), was ~25µg/50ml culture. A significant portion of the total knob protein expressed appeared to remain in a monomeric (and insoluble) form. The soluble trimeric material obtained was used for a preliminary competition experiment. Wild type Ad 2 and Ad 17 were used to infect A549 cells, or cells that had been pre-
25 incubated with excess (about 100 molecules of trimer per receptor) Ad 2 fiber knob or Ad 17 fiber knob. The results indicated that Ad 2 fiber knob, but not Ad 17 knob, could block Ad 2 infection. Additionally, Ad 17 infection was not blocked by E. coli-expressed fiber knobs of either serotype, suggesting that the mechanism of Ad 2 and Ad 17 infections is different.

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Example 6 Construction of the chimeric vector Ad2/ β gal-2/fiber Ad 17

The vector Ad2/ β gal-2 was constructed as follows. A CMV β gal expression
5 cassette was constructed in a pBR322-based plasmid that contained Ad 2 nucleotides
1-10,680 from which nucleotides 357-3328 were deleted. The deleted sequences were
replaced with (reading from 5' to 3'): a cytomegalovirus immediate early promoter
(obtained from pRC/CMV, Invitrogen), lacZ gene encoding β -galactosidase with a
nuclear localization signal, and an SV40 polyadenylation signal (nucleotides 2533-
10 2729). The resulting plasmid was used to generate Ad2/ β gal-2 by recombination with
Ad2E4ORF6 (D. Armentano et al., Human Gene Therapy, 6, 1995, pp 1343 -1353).

A chimeric Ad2/ β gal-2/fiber Ad 17 viral vector (Figure 4) was then constructed
as follows. pAdORF6 (D. Armentano et al., Human Gene Therapy, 6, 1995, pp 1343
-1353) was cut with Nde and BamHI to remove Ad 2 fiber coding and polyadenylation
15 signal sequences (nucleotides 20624-32815). An NdeI-BamHI fragment containing
Ad 17 fiber coding sequence (nucleotides 30984-32095) was generated by PCR and
ligated along with an SV40 polyadenylation signal into NdeI-BamHI cut pAdORF6 to
generate pAdORF6fiber17. This plasmid was cut with PacI and then ligated to PacI-
cut Ad2/ β gal-2 DNA to generate Ad2/ β gal-2fiber 17. Any desired transgene may be
20 substituted in this construct for the reporter gene.

A similar construct can be prepared using a DNA sequence that encodes Ad 17
penton base instead of Ad 17 fiber. Alternatively, only a subregion of the penton base
of Ad 2 need be subject to replacement, such as by inserting into the vector a
nucleotide encoding sequence corresponding to any amino acid subsequence of Ad 17
25 penton base amino acids 283-348 (see the marked sequence in Figure 5A) in
replacement for any subsequence of Ad 2 penton base amino acids 290-403.
Preferrably, the replaced sequence of Ad 2 and the inserted sequence of Ad 17
includes the RGD domain of each. Use of nucleotide sequence corresponding to
penton base amino acid sequence for other subgroup D serotypes is also within the

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practice of the invention. It is also within the scope of the invention to replace a subregion of the fiber protein in the Ad 2 vector with a subregion from another adenovirus serotype, for example, Ad 17.

5 Example 7 Ad2/ β gal-2f17 shows increased infection efficiency on human airway explants

Both human and monkey trachea explants, about 1 cm², were placed on top of an agar support. Each explant was infected at an moi of 200 of either Ad2/ β gal-2 or Ad2/ β gal-2f17 assuming a cell density of 1×10^6 per cm² of explant. Explants were
10 exposed to virus for three hours and were then rinsed with NHBE media. Two days post-infection explants were stained with X-gal and infection efficiency was assessed. On the monkey explants Ad2/ β gal-2 gave rise to a higher infection efficiency than Ad2/ β gal-2f17. Patches of stained cells were detected in explants exposed to Ad2/ β gal-2 but very few cells stained in explants exposed to Ad2/ β gal-2f17. A
15 different result was obtained on human trachea explants. On these explants Ad2/ β gal-2f17 infection gave rise to a much higher infection efficiency than Ad2/ β gal-2 infection. Approximately 5-10% of the cells in explants exposed to Ad2/ β gal-2f17 stained with X-gal whereas very few cells were stained in explants exposed to Ad2/ β gal-2. No background staining was observed in either monkey or human
20 explants that were not exposed to virus.

The results indicate that the exchange of Ad 2 fiber for Ad 17 fiber in Ad2/ β gal-2f17 was sufficient to significantly increase infection efficiency of human tracheal airway cells by an adenovirus type 2 based vector.

25 Example 8 Adenovirus subgroup screening on human cancer cell lines

Identification of adenovirus subgroup that best infects a particular tumor type may be useful in designing vectors to optimally target cancer cells in vivo. In order to determine the adenovirus subgroup that best infects a particular type of cancer cell, cancer cells were seeded into a 96 well plate and infected with and moi of 5. Infection

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efficiency was determined by staining of infected cells using an anti-hexon antibody. The adenovirus subgroups were represented by the following serotypes: A: Ad 31; B: Ad 3; C: Ad 2; D: Ad 17; E: Ad 4; and F: Ad 41.

Subgroup D (Ad 17) has a significantly higher infection rate of the colon
5 cancer cell line CaCo-2 than other cell types, with an infection rate of 70%, while Ad
2 only infected 20% of the cells (Figure 9).

Subgroup D (Ad 17) was effective in infecting ovarian cancer cell line SK-
OV3. Infection was measured at 90% (Figure 10).

10 Sequence Listing

Included herewith on the following pages are informal copies of SEQ ID NO:
1 through SEQ ID NO: 3.

1 CATCATCAAT AATATACCCC ACAAAGTAAA CAAAAGTTAA TATGCAAATG AGGTTTTTAA
61 TTTAGGGCGG GGCTACTGCT GATTGGCCGA GAAACGTTGA TGCAAATGAC GTCACGACGC
121 ACCGCTAACG GTCGCCGCGG AGGCGTGGCC TAGCCCGGAA GCAAGTCGCG GGGCTGATGA
181 CGTATAAAAA AGCGGACTTT AAACCCGGAA ACGGCCGATT TTCCCGCGGC CACGCCCGGA
241 TATGAGGTAA TTCTGGGCGG ATGCAAGTGA AATTAGGTCA TTTTGGCGCG AAAACTGAAAT
301 GAGGAAGTGA AAAGTAAAAA ATACCGGTCC CGCCCAGGGC GGAATATTTA CCGAGGGCCG
361 AGAGACTTTG ACCGATTACG TGTGGGTTTC GATTGCGGTG TTTTTTCGCG AATTTCCGCG
421 TCCGTGTCAA AGTCCGGTGT TTATGTCACA GATCAGCTGA TCCACAGGT ATTAAACCA
481 GTCGAGCCG TCAAGAGGCC ACTCTTGAGT GCCAGCGAGT AGAGATTTCT CTGAGCTCCG
541 CTCCAGAGT CTGAGAAAAA TGAGACACT GCGCCTCCTG CCTGGAACG TGCCCTTGA
601 CATGGCCGCA TTATTGCTGG ATGACTTTGT GAGTACAGTA TTGGAGGATG AAGTGAACCC
661 AACTCCGTTT GAGCTGGGAC CCACACTTCA GGACCTCTAT GATTGAGAG TAGATGCCCA
721 GGAGGACGAC CCGAACGAAG ATGCTGTGAA TTAAATATTT CCAGAATCTC TGATTCTTCA
781 GGCTGACATA GCCAGCGAAG CTCTACCTAC TCCACTTCAT ACTCCAATC TGTCACCCAT
841 ACCTGAATTG GAAGAGGAGG ACGAGTTAGA CCTCCGGTGT TATGAGGAAG GTTTTCTCTC
901 CAGCGATTCA GAGGACGAAC AGGGTGAGCA GAGCATGGCT CTAATCTCAG ACTATGCTTG
961 TGTGGTTGTG GAAGAGCATT TTGTGTTGGA CAATCCTGAG GTGCCCGGGC AAGGCTGTAA
1021 ATCCTGCCAG TACCACCGGG ATAAGACCGG AGACACGAAC GCCTCCTGTG CTCTGTGTTA
1081 CATGAAAAG AACTTCAGCT TTATTTACAG TAAGTGGAGT GAATGTGAGA GAGGCTGAGT
1141 GCTTAAGACA TAACTGGGTG ATGCTTCAAC AGCTGTGCTA AGTGTGGTTT ATTTTGTTC
1201 TAGGTCCGGT GTCAGAGGAT GGTATCACC CTCAGAAGAA GACCACCCGT GTCCCCCTGA
1261 TCTGTGAGG GAAACGCCCC TGCAAGTGCA CAGACCCACC CCAGTCAGAC CCAGTGCGA
1321 GAGGCGAGCA GCTGTTGAAA AAATTGAGGA CTGTGTACAT GACATGGGTG GGGATGAACC
1381 TTTGGACCTG AGCTTGAAC GTCCAGGAA ACTAGGCGCA GCTGCGCTTA GTCATGTGTA
1441 AATAAAGTTG TACAATAAAA ATTATATGTG ACGCATGCAA GGTGTGGTTT ATGACTCATG
1501 GCGGGGGCTT AGTTCTATAT AAGTGGCAAC ACCTGGGCAC TGGAGCACAG ACCTTCAGGG
1561 AGTTCTGAT GGATGTGTGG ACTATCCTTG CAGACTTTAG CAAGACACGC CGGCTGTAG
1621 AGGATAGTTC AGACGGGTGC TCCGGGTCTT GGAGACACTG GTTTGGAACCT CCTCTATCTC
1681 GCCTGGTGTA CACAGTTAAA AAGGATTATA ACGAGGAATT TGAAAATCTT TTTGCTGATT
1741 GCTCTGGCCT GCTAGATTCT CTGAATCTCG GCCACCAGTC CCTTTTCCAG GAAAGGGTAC
1801 TCCACAGCCT TGATTTTCC AGCCCAGGGC GCACTACAGC CGGGTTGCT TTTGTGGTTT
1861 TTCTGGTTGA CAAATGGAGC CAGAACACCC AACTGAGCAG GGGCTACATT CTGGACTTCG
1921 CAGCCATGCA CCTGTGGAGG GCATGGGTCA GGCAGCGGGG ACAGAGAATC TTGAACTACT
1981 GGCTTCTACA GCCAGCAGCT CCGGGTCTTC TTCGTCTACA CAGACAAACA TCCATGTTGG
2041 AGGAAGAAAT GAGGCAGGCC ATGGACGAGA ACCCGAGGAG CGGTCTGGAC CCTCCGTCGG
2101 AAGAGGAGTT GGATTGAATC AGGTATCCAG CCTGTACCCA GAGCTTAGCA AGGTGCTGAC
2161 ATCCATGGCC AGGGGAGTGA AGAGGGAGAG GAGCGATGGG GGCAATACCG GGATGATGAC
2221 CGAGCTGACG GCCAGTCTGA TGAATCGCAA GCGCCCAGAG CGCCTTACCT GGTACGAGCT
2281 ACAGCAGGAG TGCAGGGATG AGTTGGGCCT GATGCAGGAT AAATATGGCC TGGAGCAGAT
2341 AAAAACCCTT TGGTTGAACC CAGATGAGGA TTGGGAGGAG GCTATTAAGA AGTATGCCAA
2401 GATAGCCCTG CGCCAGATT GCAAGTACAT AGTGACCAAG ACCGTGAATA TCAGACATGC
2461 TGCTACATCT CGGGGAACGG GGCAGAGGTG GTCATTGATA CCCTGGACAA GGCCGCCTTT
2521 AGGTGTTGCA TGATGGGAAT GAGAGCCGGA GTGATGAATA TGAATTCCAT GATCTTTATG
2581 AACATGAAGT TCAATGGAGA GAAGTTTAAT GGGGTGCTGT TCATGGCCAA CAGCCACATG
2641 ACCCTGCATG GCTGCGACTT TTTCCGGCTT AACAAATATGT GCGCAGAGGT CTGGGGCGCT
2701 TCCAAGATCA GGGGATGTAA GTTTTATGGC TGCTGGATGG GCGTGGTCGG AAGACCCAAG
2761 AGCGAGATGT CTGTGAAGCA GTGTGTGTTT GAGAAATGCT ACCTGGGAGT CTCTACCGAG
2821 GGCAATGCTA GAGTGAGGCA CTGCTCTTCC CTGGAGACGG GCTGCTTCTG CCTGGTGAAG
2881 GGCACAGCCT CTCTGAAGCA TAATATGGTG AAGGGCTGCA CGGATGAGCG CATGTACAAC
2941 ATGCTGACTG CGACTCGGGG GTCTGTCATA TCCTGAAGAA CATCCATGTG ACCTCCACC
3001 CCAGAAAGAA GTGGCCAGTG TTTGAGAATA ACATGCTGAT CAAGTGCCAC ATGCACCTGG
3061 GCGCCAGAAG GGGCACCTTC CAGCCGTACC AGTGCAACTT TAGCCAGACC AAGCTGCTGT
3121 TGGAGAACGA TGCTTCTTCC AGGGTGAACC TGAACGGCAT CTTTGACATG GATGTCTCGG
3181 TGTAACAAGAT CCTGAGATAC GATGAGACCA AGTCCAGGGT GCGCGCTTGC GAGTGCGGGG
3241 GCAGACACAC CAGGATGCAG CCAGTGCCCC TGGATGTGAC CGAGGAGCTG AGACCAGACC
3301 ACCTGGTGAT GGCCTGTACC GGGACCGAGT TCAGCTCCAG TGGGGAGGAC ACAGATTAGA
3361 GGTAGGTTTG AGTAGTGGGC GTGGCTAAGG TGAATAAAA GGCGGGTGTG TTACGAGGGT

3421 CTTTTTGCTT TTCTGCAGAC ATCATGAACG GGACCGGCGG GGCCTTCGAA GGGGGGCTTT
 3481 TTAGCCCTTA TTTGACAACC CGCCTGCCAG GATGGGCGG AGTTCGTCAG AATGTGATGG
 3541 GATCGACGGT GGACGGGCGC CCAGTGCTTC CAGCAAATTC CTCGACCATG ACCTACGCGA
 3601 CCGTGGGGAA CTCGTCGCTT GACAGCACCG CCGCAGCCGC GGCAGCCGCA GCCGCCATGA
 3661 CAGCGACGAG ACTGGCCTCG AGCTACATGC CCAGCAGCAG CAGTAGCCCC TCTGTGCCCA
 3721 GTTCCATCAT CGCCGAGGAG AACTGCTGGC CCTGCTGGCC GAGCTGGAAG CCCTGAGCCG
 3781 CCAGCTGGCC GCCCTGACCC AGCAGGTGTC CGAGCTCCGC GAACAGCAGC AGCAAAATAA
 3841 ATGATTCAAT AAACACATAT TCTGATTCAA ACAGCAAAGC ATCTTTATTA TTTATTTTTT
 3901 CGCGCGCGGT AGGCCCTGGT CCACCTCTCC CGATCATTGA GAGTGCGGTG GATTTTTTCC
 3961 AAGACCCGGT AGAGGTGGGA TTGGATGTTG AGGTACATGG GCATGAGCCC GTCCCGGGGG
 4021 TGGAGGTAGC ACCACTGCAT GGCCTCGTGC TCTGGGGTCG TGTGTAGAT GATCCAGTCA
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33121 CGTGGTAAAC GGGCCCTGCG ATATGGATGA TGGCGGAGCG AGCTGGATTG AATCTCGGTT
33181 TGCATTGTAG TGGATTCTCT TGCGTACCTT GTCGTACTTC TGCCAGCAGA AATGGGCCCT
33241 TGAACAGCAT ATACCCCTCC TGCGGCCGTC CTTTCGCTGC TGCCGCTCAG TCATCCAACT
33301 GAAGTACATC CATTCTCGAA GATTCTGGAG AAGTTCCTCT GCATCTGATG AAATAAAAAA
33361 CCCGTCCATG CGAATTCCTC TCATCACATC AGCCAGGACT CTGTAGGCCA TCCCCATCCA
33421 GTTAATGCTG CCTTGTCTAT CATTAGAGG GGGCGGTGGC AGGATTGGAA GAACCATTTT
33481 TATTTCAAAC GGTCTCGAAG GACGATAAAG TGCAAGTCAC GCAGGTGACA GCGTTCCCTC
33541 CCGCTGTGCT GGTGGAACA GACAGCCAGG TCAAAACCCA CTCTATTTTC AAGGTGCTCG
33601 ACCGTGGCTT CGAGCAGTGG CTCTACGCGT ACATCCAGCA TAAGAATCAC ATTTAAAGGCT
33661 GGCCCTCCAT CGATTTCATC AATCATCAGG TTACATTCTT GCACCATCCC CAGGTAAATTC
33721 TCATTTTTC AGCCTTGGAT TATCTCTACA AATTGTTGGT GTAAATCCAC TCCGCACATG
33781 TTGAAAAGCT CCCACAGTGC CCCCTCCACT TTCATAATCA GGCAGACCTT CATAATAGAA
33841 ACAGATCCTG CTGCTCCACC ACCTGCAGCG TGTTCAAAAC AACAAAGATT AATAAGGTTT
33901 TGCCCTCCGC CCTGAGCTCG CGCCTCAATG TCAGCTGCAA AAAGTCACTT AAGTCTGGG
33961 CCACTACAGC TGACAATTCA GAGCCAGGGC TAAGCGTGGG ACTGGCAAGC GTGAGGAAAA
34021 ACTTTAATGC TCCAAAGCTA GCACCCAAAA ACTGCATGCT GGAATAAGCT CTCTTTGTGT
34081 CTCCGGTGAT GCCTTCCAAA ATGTGAGTGA TAAAGCGTGG TAGTTTTTTC TTTAATCATT
34141 TGCGTAATAG AAAAGTCTG TAAATAAGTC ACTAGGACCC CAGGGACCAC AATGTGGTAG

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34201 CTTACACCGC GTCGCTGAAA GCATGGTTAG TAGAGATGAG AGTCTGAAAA ACAGAAAGCA
34261 TGCCTAAAC TAAGGTGGCT ATTTTCACTG AAGGAAAAAT CACTCTTTCC AGCAGCAGGG
34321 TACCCACTGG GTGGCCCTTG CGGACATACA AAAATCGGTC CGTGTGATTA AAAAGCAGCA
34381 CAGTAAGTTC CTGTCTTCTT CCGGCAAAAA TCACATCGGA CTGGGTTAGT ATGTCCCTGG
34441 CATGGTAGTC ATTCAAGGCC ATAAATCTGC CCTGATATCC AGTAGGAACC AGCACACTCA
34501 CTTTtagGTG AAGCAATACC ACCCCATGCG GAGGAATGTG GAAAGATTCA GGGCAAAAAA
34561 AATTATATCT ATTGCTAGCC CTTCTGGAC GGGAGCAATC CTCCAGGACT ATCTATGAAA
34621 GCATACAGAG ATTCAGCCAT AGCTCAGCCC GCTTACCAGT AGACAAAGAG CACAGCAGTA
34681 CAAGCGCCAA CAGCAGCGAC TGACTIONCA CTGACTTAGC TCCCTATTTA AAGGCACCTT
34741 AACTGACGT AATGACCAAA GGTCTAAAAA CCCC GCCAAA AAAACACACA CGCCCTGGGT
34801 GTTTTTCGA AAACACTTCC GCGTCTCAC TTCCTCGTAT CGATTTCGTG ACTTGACTTC
34861 CGGGTTCCCA CGTTACGTCA CTTTGGCCCT TACATGTAAC TTAGTCGTAG GCGCCATCT
34921 TGCCACGTC CAAAATGGCT TACATGTCCA GTTACGCCTC CGCGGCGACC GTTAGCCGTG
34981 CGTCGTGACG TCATTGTCAT CAACGTTTCT CGGCAATCA GCAGTAGCCC CGCCCTAAAT
35041 TAAAAACCTC ATTTGCATAT TAACTTTTGT TTACTTTGTG GGTATATTA TTGATGATG

ATGTCAAAGAGGCTCCGGGTGGAAGATGACTTCAACCCCGTCTACCCCTA
TGGCTACGCCGCGGAATCAGAATATCCCCTTCCTCACTCCCCCCTTTGTCTC
CTCCGATGGATTCAAAAACCTTCCCCCCTGGGGTCCTGTCACTCAAACCTGGC
TGACCCAATCACCATAGCCAATGGTGATGTCTCACTCAAGGTGGGAGGGG
GACTTACTTTGCAAGAAGGAAGTCTGACTGTAGACCCTAAGGCTCCCTTG
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GAAGGATTCAAAACCTCACTTTGGTACTTACAAAGTGTGGAAGTCAAATAT
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ACGCTACAAATCCAACCTCTTAAATCATTTAAAATAAACTTCTTTTTGATA
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TTAGAAGTGACAATTTAACTGTATCTGAGGCATATAAAAATGCAGTTGAA
TTTATGCCTAATTTGGTAGCCTACCCAAAACCTACCACTGGCTCTAAAAAA
TATGCAAGGGATATAGTCTATGGGAACATATATCTTGGAGGTTTGGCATA
TCAGCCAGTTGTAATTAAGGTTACTTTTAATGAAGAAGCAGATAGTGCTTA
CTCTATAACATTTGAATTTGTATGGAATAAAGAATATGCCAGGGGTTGAA
TTTGAAACCACTTCCTTTACCTTCTCCTATATTGCCCAACAATAA

SEQ ID NO:2

SUBSTITUTE SHEET (RULE 26)

Penton17.Seq Length: 1554

```
1  ATGAGGCGTG CGGTGGTGTC TTCCTCTCCT CCTCCCTCGT ACGAGAGCGT
51  GATGGCGCAG GCGACCCTGG AGGTTCGGTT TGTGCCTCCG CGGTATATGG
101 CTCCTACGGA GGGCAGAAAC AGCATTCGTT ACTCGGAGCT GGCTCCGTTG
151 TACGACACCA CTCGCGTGTA CTTGGTGGAC AACAAAGTCGG CGGACATCGC
201 TTCCCTGAAC TATCAAAACG ACCACAGCAA CTTCCCTGACC ACGGTGGTGC
251 AGAACAAACG TTTCACCCCC GCCGAGGCTA GCACGCAGAC GATAAAATTT
301 GACGAGCGGT CGCGGTGGGG CGGTGATCTG AAGACCATTC TGCACACCAA
351 CATGCCCAAT GTGAACGAGT ACATGTTTAC CAGCAAGTTT AAGGCGCGGG
401 TGATGGTGGC TAGAAAACAC CCACAGGGGG TAGAAGCAAC AGATTTAAGC
451 AAGGATATCT TAGAGTATGA GTGGTTTGAG TTTACCCTGC CCGAGGGCAA
501 CTTTTCGAG ACCATGACCA TAGACCTGAT GAACAACGCC ATCTTGAAA
551 ACTACTTGCA AGTGGGGCGG CAAAATGGCG TGCTGGAGAG CGATATTGGA
601 GTCAAGTTTG ACAGCAGAAA TTTCAAGCTG GGCTGGGACC CTGTGACCAA
651 GCTGGTGATG CCAGGGGTCT ACACCTACGA GGCTTTTAC CCGGACGTGG
701 TGCTGCTGCC GGGCTGCGGG GTGGACTTCA CAGAGAGCCG CCTGAGCAAC
751 CTCCTGGGCA TTCGCAAGAA GCAACCTTTC CAAGAGGGCT TCAGAATCAT
801 GTATGAGGAT CTAGAAGGGG GCAACATCCC CGCCCTGCTG GATGTGCCCA
851 AGTACTTGGA AAGCAAGAAG AAGTTAGAGG AGGCATTGGA GAATGCTGCT
901 AAAGCTAATG GTCCTGCAAG AGGAGACAGT AGCGTCTCAA GAGAGGTTGA
951 AAAGGCAGCT GAAAAAGAAC TTGTTATTGA GCCCATCAAG CAAGATGATA
1001 CCAAGAGAAG TTACAACCTC ATCGAGGGAA CCATGGACAC GCTGTACCGC
1051 AGCTGGTACC TGTCTTATAC CTACCGGGAC CCTGAGAACG GGGTGCAGTC
1101 GTGGACGCTG CTCACCACCC CGGACGTCAC CTGCGGCGCG GAGCAAGTCT
1151 ACTGGTCGCT GCCGGACCTC ATGCAAGACC CCGTCACCTT CCGTTCTACC
1201 CAGCAAGTCA GCAACTACCC CGTGGTCGGC GCCGAGCTCA TGCCCTTCCG
1251 CGCCAAGAGC TTTTACAACG ACCTCGCCGT CTACTCCCAG CTCATCCGCA
1301 GCTACACCTC CCTCACCCAC GTCTTCAACC GCTTCCCCGA CAACCAGATC
```

SEQ ID NO: 3

1351 CTCTGCCGTC CGCCCGCGCC CACCATCACC ACCGTCAGTG AAAACGTGCC
1401 TGCTCTCACA GATCACGGGA CGCTACCGCT GCGCAGCAGT ATCCGCGGAG
1451 TCCAGCGAGT GACCGTCACT GACGCCCGTC GCCGCACCTG TCCCTACGTC
1501 TACAAGGCCC TGGGCATAGT CGCGCCGCGT GTGCTTTCCA GTCGCACCTT
1551 CTAA

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Claims

1. A chimeric adenoviral vector comprising nucleotide sequence of a first
adenovirus, wherein at least one gene of said first adenovirus encoding a
protein that facilitates binding of said vector to a target mammalian cell, or
internalization thereof within said cell, is replaced by the corresponding gene
from a second adenovirus belonging to subgroup D, said vector further
comprising a transgene operably linked to a eucaryotic promoter to allow for
expression therefrom in a mammalian cell.
2. A chimeric adenoviral vector according to Claim 1 wherein said second
adenovirus is selected from the group consisting of Ad 9, Ad 15, Ad 17, Ad
19, Ad 20, Ad 22, Ad 26, Ad 27, Ad 28, Ad 30, and Ad 39.
3. A chimeric adenoviral vector according to Claim 1 wherein said first
adenovirus is selected from the group consisting of Ad 2, Ad 5, and Ad 12.
4. A chimeric adenoviral vector according to Claim 1 wherein said replaced gene
encodes Ad fiber.
5. A chimeric adenoviral vector according to Claim 1 wherein said replaced gene
encodes Ad penton base.
6. A chimeric adenoviral vector according to Claim 1 wherein a first replaced
gene encodes Ad fiber, and a second replaced gene encodes Ad penton base.
7. A chimeric adenoviral vector comprising nucleotide sequence of a first
adenovirus, wherein a portion of a gene thereof encoding a protein that
facilitates binding of said vector to a target mammalian cell, or internalization

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thereof within said cell, is replaced by a portion of the corresponding gene from a second adenovirus belonging to subgroup D, said vector further comprising a transgene operably linked to a eucaryotic promoter to allow for expression therefrom in a mammalian cell.

5

8. A chimeric adenoviral vector according to Claim 7 wherein the encoding sequence that is replaced codes for a portion of Ad fiber.

9. A chimeric adenoviral vector according to Claim 7 wherein the encoding
10 sequence that is replaced codes for a portion of Ad penton base.

10. A chimeric adenoviral vector according to Claim 9 wherein the encoding sequence that is replaced codes for an amino acid sequence that includes RGD.

15 11. A method of providing a biologically active protein to the airway epithelial cells of a patient comprising administering to said cells an adenoviral vector selected from the group consisting of:

(a) a chimeric adenoviral vector comprising nucleotide sequence of a first adenovirus, wherein at least one gene of said first adenovirus
20 encodes a protein that facilitates binding of said vector to a target mammalian cell, or internalization thereof within said cell, is replaced by the corresponding gene from a second adenovirus belonging to subgroup D, said vector further comprising a transgene encoding said protein that is operably linked to a eucaryotic promoter to allow for
25 expression therefrom in a mammalian cell; and

(b) a chimeric adenoviral vector comprising nucleotide sequence of a first adenovirus, wherein a portion of a gene thereof encoding a protein that facilitates binding of said vector to a target mammalian cell, or internalization thereof within said cell, is replaced by a portion of the

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corresponding gene from a second adenovirus belonging to subgroup D, said vector further comprising a transgene encoding said protein that is operably linked to a eucaryotic promoter to allow for expression therefrom in a mammalian cell;

5 under conditions whereby the transgene encoding said protein is expressed, and phenotypic benefit is produced in said airway epithelial cells.

12. A method according to Claim 11 wherein said second adenovirus is Ad 17 and the nucleotide sequence thereof used in replacement of nucleotide sequence of
10 said first adenovirus encodes a polypeptide selected from the group consisting of Ad 17 fiber, a fragment of Ad 17 fiber, Ad 17 hexon, a fragment of Ad 17 hexon, Ad penton base, and a fragment of Ad 17 penton base.

13. A method of providing a biologically active protein to the airway epithelial
15 cells of a patient that comprises administering to said cells an adenoviral vector comprising elements of an Ad 17 genome, and a transgene encoding said protein that is operably linked to a eucaryotic promoter to allow for expression therefrom in a mammalian cell, under conditions whereby the transgene encoding said protein is expressed, and phenotypic benefit is
20 produced in said airway epithelial cells.

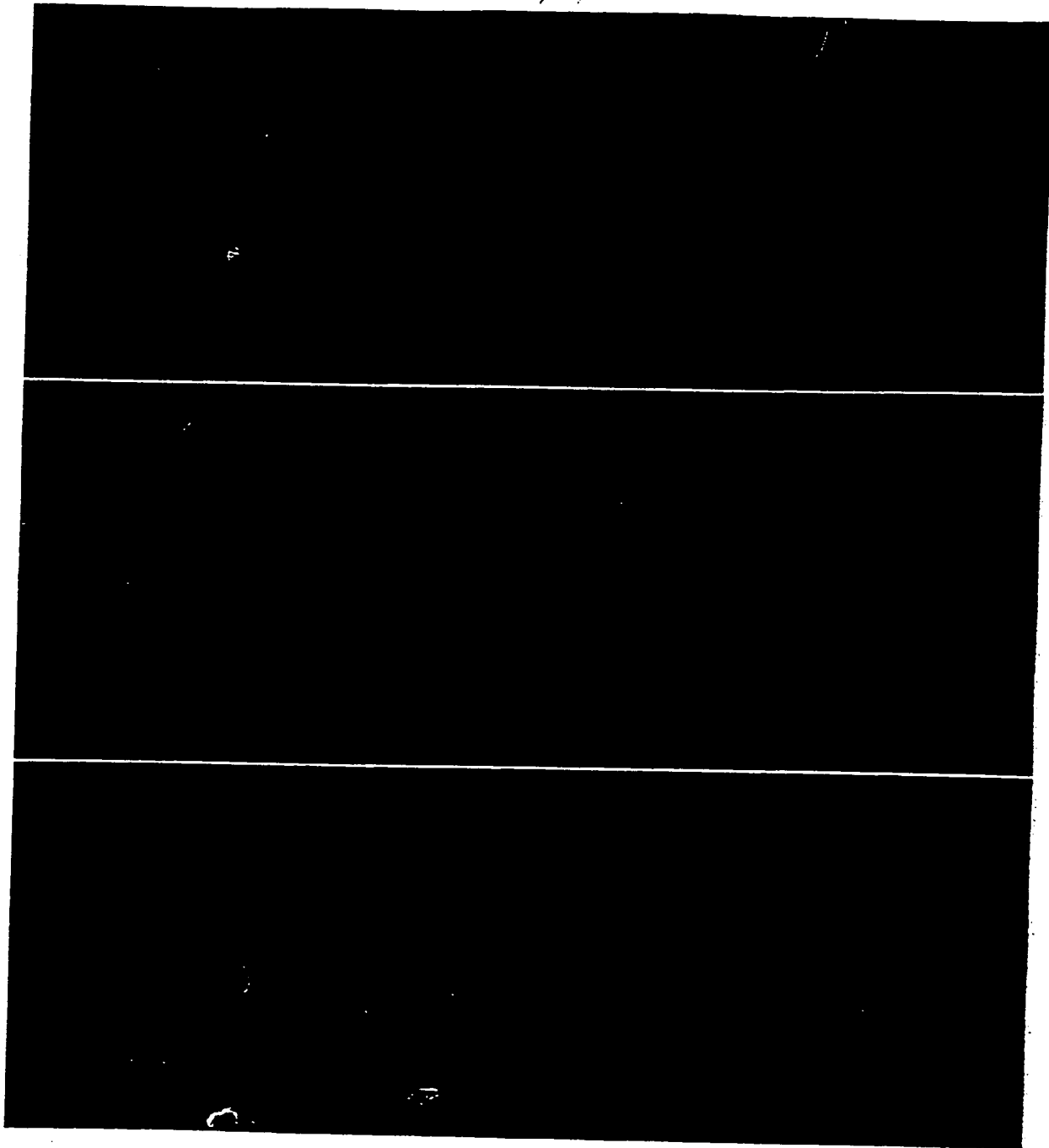


FIG 1 - original FIG 1 is PTO
is full color - see side Solder

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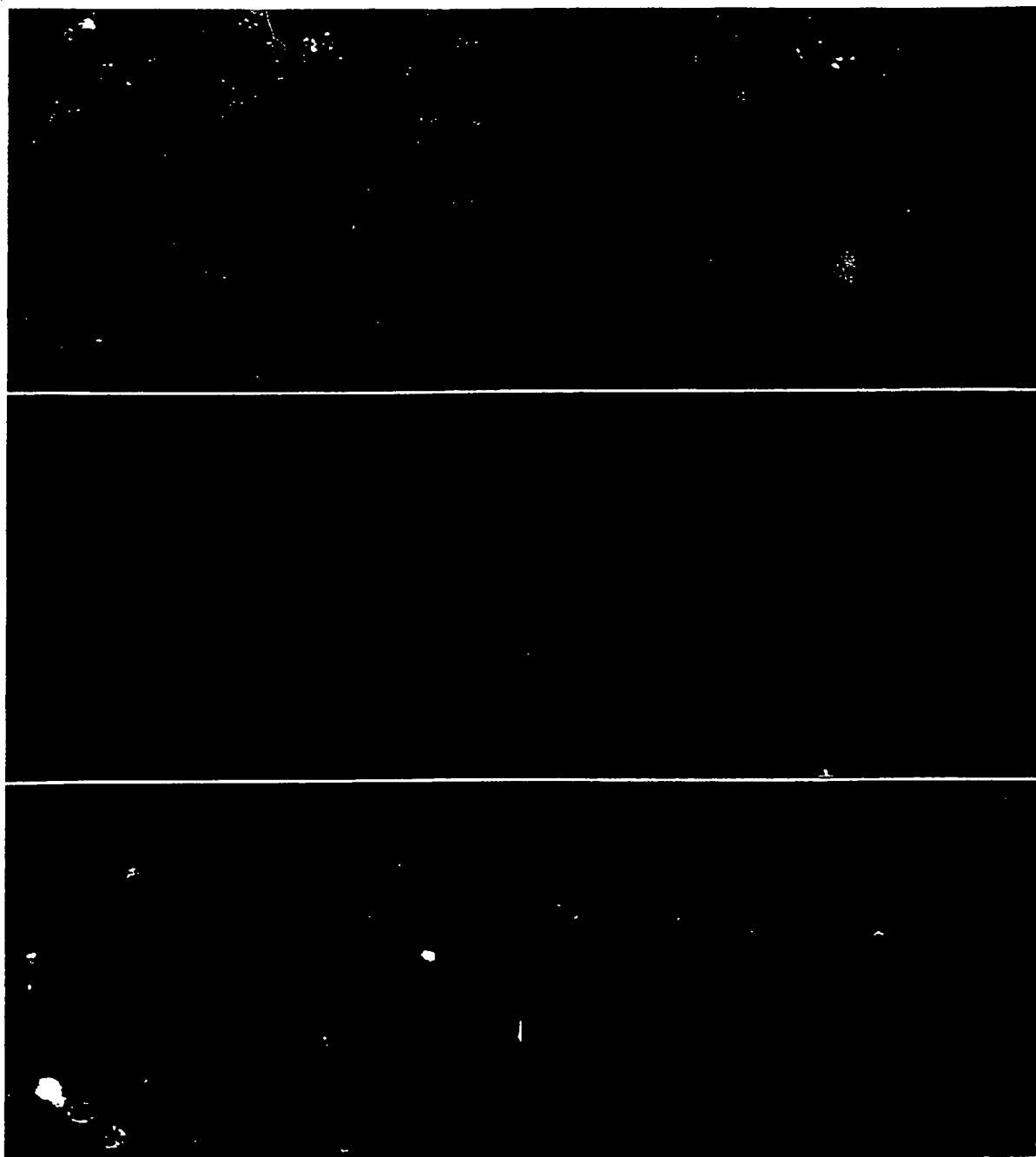


FIG 2 - original filed in PTO
is full color - see side folder

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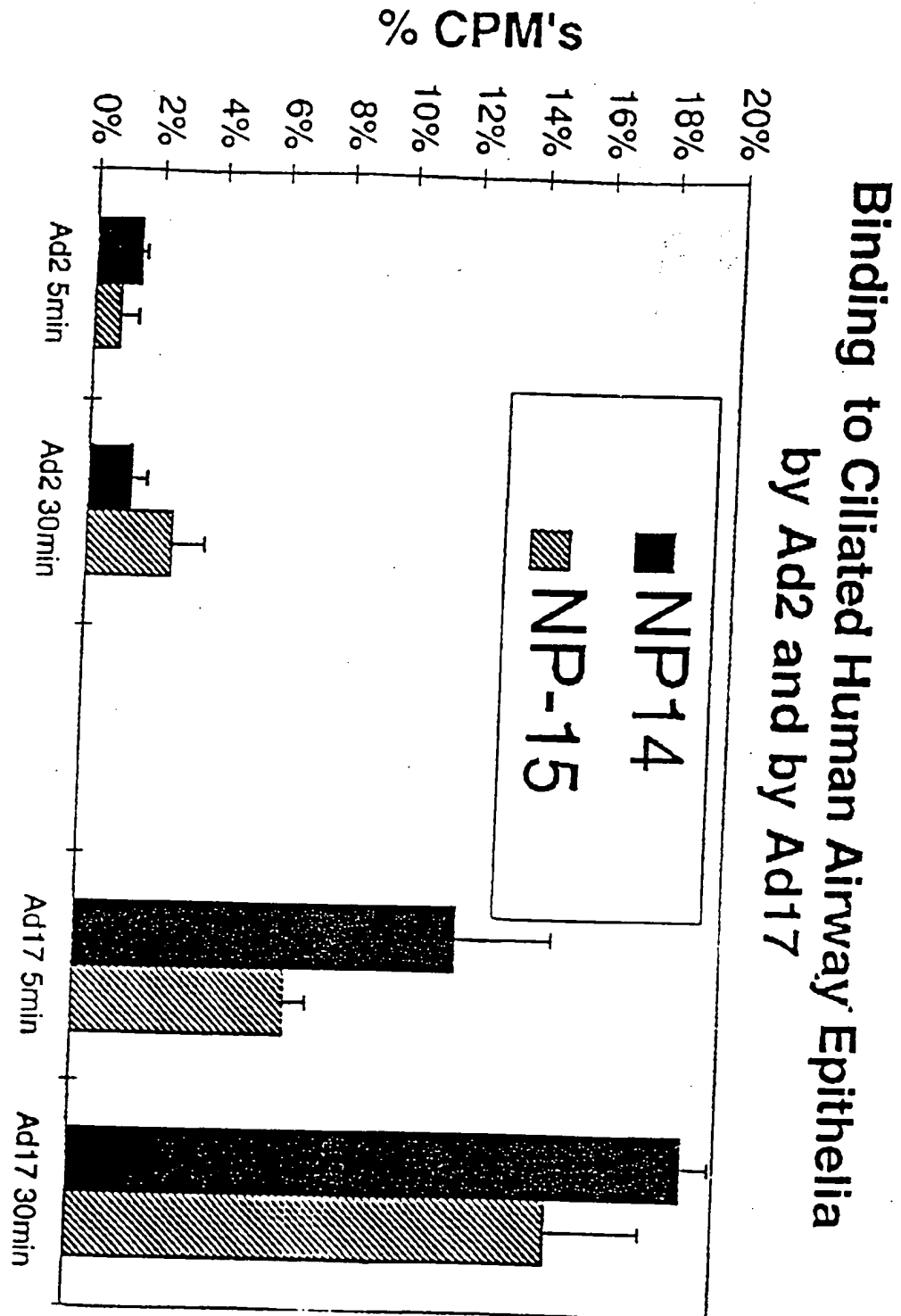


FIGURE 3

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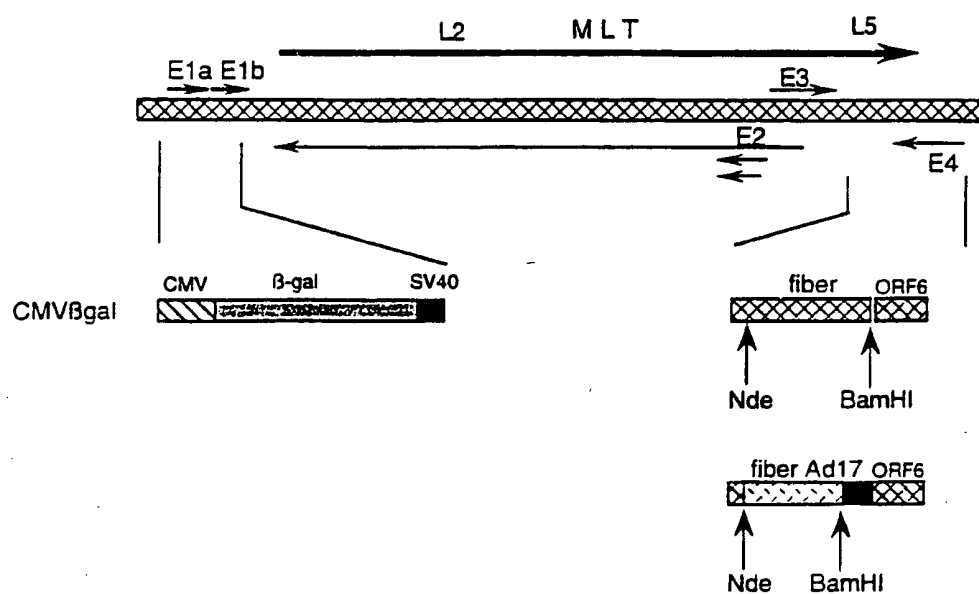
Chimeric Ad2/ β gal-2/ Ad17 vectors

FIGURE 4

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[illegible]

END

FIGURE 5A

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393 DPVTFRSTQQVSNYPVVGAELMPFRAKSFYNDLAVYSQLIRSYTSLTHVF 442
||||| :||:|||||:|.:.||||| ||||| :|||||
447 DPVTFRSTQISNFPVVGAELLPVHKSIFYNDQAVYSQLIRQFTSLTHVF 496
443 NRFPDNQILCRPPAPTITTVSENPALTDHGTLPLRSSIRGVQRVTVTDA 492
|||:||||.||||| ||||| ||||| ||||| :|||
497 NRFPENQILARPPAPTITTVSENPALTDHGTLPLRNSIGGVQRVTTTDA 546
493 RRRTCPYVYKALGIVAPRVLSSRTF 517
||||| |||||
547 RRRTCPYVYKALGIVSPRVLSSRTF 571

FIGURE 5B

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//

	1		50	
Penton5	...MRRAM.YEEGP	PPSYESVUSA ..APVAAALG	SPFDAPLDPP	← SEQ ID NO: 6
Penton2	...MQRAAM.YEEGP	PPSYESVUSA ..APVAAALG	SPFDAPLDPP	← SEQ ID NO: 5
Penton3	...MRRRAVLG	GAV.VYPEGP PPSYESVM..QQQA AMIQPFLEAP	← SEQ ID NO: 7
Penton12	...MRRRAVEL	QTV.AFPETP PPSYETVM..AAAPP	↑ SEQ ID NO: 8
Penton40	...MRRRAVG	PPVMAYAE GP PPSYESVM..ET ADLPATLQAL	↑ SEQ ID NO: 9
Penton17	...MRRRAVV.SSSP	PPSYESVM..A.... QATLEVP	↑ SEQ ID NO: 4
Pentonf10	MWGLQPPTSI	PPPPPTTELT PSTYPAMVNG	YPPPAASAQS CSSSGGQSEL	↑ SEQ ID NO: 10
	51		100	
Penton5	FVP.PRYLRP	TGGRNSIRYS ELAPLFDTTR	VYLVDNKSTD VASLNYQNDH	
Penton2	FVP.PRYLRP	TGGRNSIRYS ELAPLFDTTR	VYLVDNKSTD VASLNYQNDH	
Penton3	FVP.PRYLAP	TEGRNSIRYS DVSPLYDTTK	LYLVDNKSAD IASLNYQNDH	
Penton12	YVP.PRYLGP	TEGRNSIRYS ELSPLYDTTR	VYLVDNKSSD IASLNYQNDH	
Penton40	HVP.PRYLGP	TEGRNSIRYS ELAPLYDTTR	VYLVDNKSAD IASLNYQNDH	
Penton17	FVP.PRYMAP	TEGRNSIRYS ELAPLYDTTR	VYLVDNKSAD IASLNYQNDH	
Pentonf10	YMLQVRVMA	TGGRNSIKYR DYTPCRNTTK	LFYVDNKASD IDTYNKDANH	
	101		150	
Penton5	SNFLTTVIQN	NDYSPGEAST QTINLDRSH	WGGDLKTILH TNMPNVNEFM	
Penton2	SNFLTTVIQN	NDYSPGEAST QTINLDRSH	WGGDLKTILH TNMPNVNEFM	
Penton3	SNFLTTVQON	NDFTPTPEAST QTINFDESR	WGGQLKTIH TNMPNVNEYM	
Penton12	SNFLTTVQON	NDYSPGEAST QTINFDESR	WGGDLKTILH TNMPNVNDFM	
Penton40	SNFOTTVQON	NDFTPTPEAST QTINFDESR	WGGDLKTILR TNMPNVNEFM	
Penton17	SNFLTTVQON	NDFTPAEAST QTINFDESR	WGGDLKTILH TNMPNVNEYM	
Pentonf10	SNFRTTVIHN	QDLADTAAT ESIQLDNRS	C WGGDLKTAVR TNCNVSSFF	
	151		200	
Penton5	FTNKFKARVM	VSRL..... PTKD..N	QVELKYEWFE FTLPEGNYSE	
Penton2	FTNKFKARVM	VSRS..... LTKD..K	QVELKYEWFE FTLPEGNYSE	
Penton3	FSNKFKARVM	VSRRKPEGV	VNDTYDH..K EDILKYEWFE	FILPEGNFSA
Penton12	FTTKFKARVM	VARK..... TNNE..G	QTILEYEWAE FVLPEGNYSE	
Penton40	STNKFKARVM	VEK..... VNR..K	TNAPRYEWFE FTLPEGNYSE	
Penton17	FTSKFKARVM	VARKHPOGV. ..EATDL..S	KDILEYEWFE FTLPEGNFSE	
Pentonf10	QSNSVRVRM	WKRDPPTSTA	PPSAVGSYS VPGAQYKGYD	LTVPEGNYAL
	201		250	
Penton5	TMTIDLMNA	IVEHYLKVGR ONGVLES	DIG VKFDTRNFRL	GFDPTGLVM

FIGURE 6A

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Penton2	TMTIDLMNNA	IVEHYLA.GR	QNGVLESDIG	VKFDTRNFRL	GFDPVTLGLVM
Penton3	TMTIDLMNNA	IIDNYLEIGR	QNGVLESDIG	VKFDTRNFRL	GWDPETKLIM
Penton12	TMTIDLMNNA	IIHYLRVGR	QHGVSLESDIG	VKFDTRNFRL	GWDPETQLVT
Penton40	TMTIDLMNNA	IVDNYLAVGR	QNGVLESDIG	VKFDTRNFRL	GWDPVTKLVM
Penton17	TMTIDLMNNA	ILENYLOVGR	QNGVLESDIG	VKFDSRNFKL	GWDPVTKLVM
Pentonf10	CELIDLLNEG	IVQLYLSEGR	QNNVQKSDIG	VKFDTRNFGL	LRDPVTGLVT
251 300					
Penton5	PGVYTNEAFH	PDIILLPGCG	VDFTHSRLSN	LLGIRKRQPF	QEGFRITYDD
Penton2	PGVYTNEAFH	PDIILLPGCG	VDFTHSRLSN	LLGIRKRQPF	QEGFRITYDD
Penton3	PGVYTYEAFH	PDIIVLLPGCG	VDFTESRLSN	LLGIRKRHPF	QEGFKIMYED
Penton12	PGVYTNEAFH	PDIIVLLPGCG	VDFTESRLSN	LLGIRKRQPF	QEGFVIMYEH
Penton40	PGVYTNEAFH	PDIIVLLPGCG	VDFTQSRLNN	LLGIRKRMPF	QKGFQIMYED
Penton17	PGVYTYEAFH	PDIIVLLPGCG	VDFTESRLSN	LLGIRKRQPF	QEGFRIMYED
Pentonf10	PGTYVYKGYH	PDIIVLLPGCA	IDFTYSRLSL	LLGIGKREPY	SKGFVITYED
301 350					
Penton5	LEGGNIPALL	DVDAYQASLK	DDTEQGGGGA	GGSNSSGSGA	EENSNAAAAA
Penton2	LEGGNIPALL	DVDAYQASLK	DDTEQGGGGA	GGGNSSGSGA	EENSNAAAAA
Penton3	LEGGNIPALL	DVTAYEESKK	DTTETTTLA	VAEETSE...
Penton12	LEGGNIPALL	DVKKYENSL	Q...
Penton40	LEGGNIPALL	DVEKYEASIK
Penton17	LEGGNIPALL	DVPKYLESKK	KLE.....E	ALENAAK...
Pentonf10	LQGGDIPALL	DLDSVDVND	DGEVIELDNA	A.....
351 400					
Penton5	MQPVEDMNDH	AIRGDTFATR	AEEKRAEAEA	AAEAAAPAAQ	PEVEKPQKKP
Penton2	MQPVEDMNDH	AIRGDTFATR	AEEKRAEAEA	AAEAAAPAAQ	PEVEKPQKKP
Penton3DDD	ITRGDTYITE	KOKREAAAAE	V.....KKEL
Penton12DQN	TVRGDNFIA	L.....NKAA
Penton40EAQ	EIRGADFKPN	PQ.....DL
Penton17ANG	PARGDSSVSR	EVEKAA.....EKEL
Pentonf10
401 450					
Penton5	VIKPLTEDSK	KRSYNLI...	SNDSTFTQYR	SWYLAYNYGD	PQTGIRSWTL
Penton2	VIKPLTEDSK	KRSYNLI...	SNDSTFTQYR	SWYLAYNYGD	PQTGIRSWTL
Penton3	KIOPLEKDSK	SRSYNVL...	E.DKINTAYR	SWYLSYNYGN	PEKGIRSWTL
Penton12	RIEPVETDPK	GRSYNLL...	P.DKINTKYR	SWYLAYNYGD	PEKGVRSWTL
Penton40	EIVPVEKDSK	ERSYNLL...	EGDKINTAYR	SWFLAYNYGD	AEKGVKSWTL
Penton17	VIEPIKQDDT	KRSYNLI...	E.GTMDTLYR	SWYLSYTYRD	PENGVOQSWTL
Pentonf10	...PLLHDSA	GVSYNVIYDQ	VTGKPVYAYR	SWMLAYNVPN	SQANQT...TL
451 500					
Penton5	LCTPDVTCGS	EQVYWSLPDM	MODPVTFRST	RQISNFPVVG	AELLPVHSKS
Penton2	LCTPDVTCGS	EQVYWSLPDM	MODPVTFRST	SQISNFPVVG	AELLPVHSKS
Penton3	LTTSDVTCGA	EQVYWSLPDM	MODPVTFRST	RQVNNYFVVG	AELMPVPSKS
Penton12	LTTSDVTCGS	EQVYWSLPDM	MODPVTFRST	RQVSNYFVVA	AELLPVHAKS
Penton40	LTTSDVTCGS	EQVYWSLPDM	MODPVTFRPS	TQVSNYFVVG	VELLPVHAKS
Penton17	LTTSDVTCGA	EQVYWSLPDL	MODPVTFRST	QQVSNYFVVG	AELMPFRKAS
Pentonf10	LTVPDMAGGI	GAMYTSLPDT	FIAPTGFKEG	NTTNLCFVVG	MNLFPTYNKI
501 550					
Penton5	FYNQAVYSQ	LIRQFT.SLT	HVFNRFPENQ	ILARPPAPTI	TTVSENVPAL
Penton2	FYNQAVYSQ	LIRQFT.SLT	HVFNRFPENQ	ILARPPAPTI	TTVSENVPAL
Penton3	FYNEQAVYSQ	QLRQAT.SLT	HVFNRFPENQ	ILIRPPAPTI	TTVSENVPAL
Penton12	FYNEQAVYSQ	LIRQST.ALT	RVFNRFPENQ	ILVRPPAATI	TTVSENVPAL

FIGURE 6B

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Penton40 FYNEQAVYSQ LIRQST.ALT HIFNRFPENQ ILVRPPAPTI TTVSENVFAL
Penton17 FYNDLAVYSQ LIRSYT.SLT HVFNRFDPNQ ILCRPPAPTI TTVSENVFAL
Pentonf10 YYQAASTYVQ RLENSCQSAT AAFNRFPENE ILKQAPPMNV SSVCDNQPAV

551

600

Penton5 TDHGTLP LRN SIGGVQRTI TDARRRTPY VYKALGIVSP RVLSSRTF*.
Penton2 TDHGTLP LRN SIGGVQRTI TDARRRTPY VYKALGIVSP RVLSSRTF*.
Penton3 TDHGTLP LRS SIRGVQRTV TDARRRTPY VYKALGIVAP RVLSSRTF*.
Penton12 TDHGTLP LRS SISGVQRTI TDARRRTPY VYKALGIVSP RVLSSRTF*.
Penton40 TDHGTLP LRS SISGVQRTI TDARRRTPY VHKALGIVAP KVLSSRTF*.
Penton17 TDHGTLP LRS SIRGVQRTV TDARRRTPY VYKALGIVAP RVLSSRTF*.
Pentonf10 VQQGVLPVKS SLPGLQRVLI TDDQRRPIPY VYKSIATVQP TVLSSATLQ*

FIGURE 6C

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Fiber17.Pep x Fiber2.Pep

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1 MSKRLRVEDDFNPVYPYGYARN.QNIPFLTPPFVSSDGFKNFPFPGVLSLK 49 ← SEQ ID:11
1 MKRARPSDDTFNPVYPYDTETGPPTVPFLTPPFVSPNGFQESPPGVLSLR 50 ← SEQ ID NO:12
50 LADPITIANGDVSLKVGGGLTQE..... 73
51 VSEPLDTSHGMLALKMGSGTLTDKAGNLTSQNVTTVTQPLKKTksNISLD 100
74 .....GSLTVDPKAPLQLA.....NNKKLELVYVDPF 100
101 TSAPLTITSGALTVATTAPLIVTSGALSVQSQAPLTVQDSKLSIATKGPI 150
101 EVSANKLSLKVGHGLK.....ILDDK 121
151 TVSDGKLALQTSAPLSGSDSDLTVTASPLTTATGSLGINMEDPIYVNN 200
122 SAGGLK.....DLIGKLVVLTGKGIGTE..... 144
201 GKIGIKISGPLQVAQNSDTLTVVTGPGVTVEQNSLRTKVAGAIGYDSSNN 250

145 .....NLQNTD...GSSRGIGISVRARE 164
301 YNRGLYLFNASNNTKKLEVSIIKKSSGLNFDNTAIAINAGKLEFDINTSE 350
165 .....GLTFDNDGYLVAWNPKYDTRT 185
351 SPDINPIKTKIGSGIDYNENGAMITKLGAGLSFDNSGAITIGNKNDDKLT 400
186 LWTTPTDSPNCRIDKEKDSKLTVLTKCGSQILANVSLIVVSGKYQYIDH 235
401 LWTTPDPSPCRIHSDNDCKFTLVLTCKGSQVLATVAALAVSGDLS.... 446

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FIGURE 7A

```

236 ATNPTLKSFKIKLLFDNKGVLPPSSNL DSTYWNFRSDNLTVSEAYKNAVE 285
    . . . | : | . . | | | . . | L P S S N L D S T Y W N F R S D N L T V S E A Y K N A V E
447 SMTGTVASVSIFLRFDONGVLMENSSLKKHYWNRNGNSTNANPYTNAV G 496
    . . . | : | . . | | | . . | S L K K H Y W N R N G N S T N A N P Y T N A V G
286 FMPN LVAYPKPTTGSKKYARDIVYGNIYLGLLAYQPVVIKVTFNEEAD.. 333
    | | | | : | | | . . . | : : : : | | | . . . | : : : : | : : :
497 FMPN LLAYPKQTQSQT...AKNNIVSQVYLHGDKTKPMILTITLNGTSEST 543
    . . . | : | | . . . | . . . | : : : : | : : : : | : : :
334 .....SAYSITFEFVNKE.YARVEFETTSFTFSYIAQQ 366
    . | : | : | . : | : . | . . | : | : | : | : | :
544 ETSEVSTYSMSFTWSWESGKYTTETFATNSYTF SYIAQE 582
    . . . | : | : | . : | : . | . . | : | : | : | : | :

```

FIGURE 7B

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	1				50
8fiber	MTKRLRA...EDDFN	PVYPYGYARN	Q.NIPFLTPP	FVSSNGFQNF - SEQ ID NO:13
9fiber	MSKRLRV...EDDFN	PVYPYGYARN	Q.NIPFLTPP	FVSSDGFQNF - SEQ ID NO:14
15fiber	MSKRLRV...EDDFN	PVYPYGYARN	Q.NIPFLTPP	FVSSDGFQNF - SEQ ID NO:15
17fiber	MSKRLRV...EDDFN	PVYPYGYARN	Q.NIPFLTPP	FVSSDGFQNF - SEQ ID NO:11
2fiber	.MKRARP...SEDTFN	PVYPYDTETG	PPTVPFLTPP	FVSPNGFQES - SEQ ID NO:12
5fiber	.MKRARP...SEDTFN	PVYPYDTETG	PPTVPFLTPP	FVSPNGFQES - SEQ ID NO:16
4fiber	MSKSARG...WSDGFD	PVYPYDADND	RP.CPSSTLP	SFSSDGFQEK - SEQ ID NO:17
40-1fiber	.MKRTRIE...DDFN	PVYPYD.TSS	TPSIPYVAPP	FVSSDGLQEN - SEQ ID NO:18
41fiber	.MKRTRIE...DDFN	PVYPYD.TFS	TPSIPYVAPP	FVSSDGLQEK - SEQ ID NO:19
40-2fiber	.MKRARFE...DDFN	PVYPYE.HYN	PLDIPFITPP	FASSNGLQEK - SEQ ID NO:20
12fiber	.MKRSRTQYA	EETEENDDFN	PVYPFD.PFD	TSVVPFVTPP	FTSSNGLQEK - SEQ ID NO:21
3fiber	MAKRARL...STSFN	PVYPYEDESS	SOH.PFINPG	FISPDGFTQS - SEQ ID NO:22
	51				100
8fiber	PPGVLSLKLA	DPITIN.NGN	VSLKVGGGLT	LQEET.....
9fiber	PPGVLSLKLA	DPIAIV.NGN	VSLKVGGGLT	LQDGT.....
15fiber	PPGVLSLKLA	DPIAIA.NGN	VSLKMGGGLT	LQEGT.....
17fiber	PPGVLSLKLA	DPITIA.NGD	VSLKVGGGLT	LQE.....
2fiber	PPGVLSLRVS	EPLDTS.HGM	LALKMGSGLT	LDKAGNLTQ	NVTTVTQPLK
5fiber	PPGVLSLRVS	EPLVTS.NGM	LALKMGNGLS	LDEAGNLTQ	NVTTVSPPLK
4fiber	PLGVLSLPG	RPCHTK.NGE	ITLKLGEVD	LDDSGKLIAN	TVNKAIAPL.
40-1fiber	PPGVLALKYT	DPITTNAKHE	LTCLKGSNIT	LQ.NGLLSA.
41fiber	PPGVLALKYT	DPITTNAKHE	LTCLKGSNIT	LE.NGLLSA.
40-2fiber	PPGVLSLKYT	DPLTTK.NGA	LTCLKGTGLN	IDKNGDLSSD	ASVEVSAPIT
12fiber	PPGVLALNYK	DPIVTE.NGT	LTCLKGDGK	LNAQGQLTAS	NNINVLEPLT
3fiber	PNGVLSLKCV	NPLTTA.SGS	LQKVGSGLT	VD.....
	101				150
8fiber
9fiber
15fiber
17fiber

FIGURE 8A

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2fiber	KTKSNISLD.	S	LTITSGA	LTVATTAPLI	VTSGALS	QAPLTVODSK
5fiber	KTKSNINLEI	SAPLTVTSEA	LTVAAAAPLM	VAGNTLTMQS	QAPLTVHDSK	
4fiberSFFQQH	HFPL.....	
40-1fiber	
41fiber	
40-2fiber	KTNKIVGLNY	TKPLALQNN	LTLNTRAPLT	TTDESLALIT	APPITVESSR	
12fiber	NTSQGLKLSW	SAPLAVKASA	LTLNTRAPLT	TTDESLALIT	APPITVESSR	
3fiber	
	151					200
8fiber	
9fiber	
15fiber	
17fiber	
2fiberLSI	
5fiberLSI	
4fiber	
40-1fiber	
41fiber	
40-2fiber	NANNELSL	IDAPLNADTG	TLRLRSDAPL	GLVDK.TLKV	
12fiber	LGLATIAPLS	LDGGGNLGLN	LSAPLDVSNN	NLHLTTETPL	VVNSSGALS	
3fiber	
	201					250
8fiberGKLT	VNTEPPLH..	
9fiberGKLT	VNADPPLQ..	
15fiberGNLT	VNTEPPLQ..	
17fiberGSLT	VDPKAPLQ..	
2fiber	ATKGPIVSD	GKLALQTSAP	LSGSDSDTLT	VTASPPPLTTA	TGSLGINMED	
5fiber	ATQGPLTVSE	GKLALQTSAP	LTTDSSTLT	ITASPPPLTTA	TGSLGIDLKE	
4fiberTWIP	LYTPKMNYP	YKFLPPLSIL	KSTI.....	
40-1fiberTVPT...VSPPLTNS	NNSLGLATSA	
41fiberTVPT...VSPPLTNS	NNSLGLATSA	
40-2fiber	LFSSPLYLDN	NFLTLAIERP	LALSSNRAVA	LKYSPPPLKIE	NENLTLSTGG	
12fiber	ATADPISVRN	NALTLPTADP	LMVSSD.GLG	ISVTSPITVI	NGSLALSTTA	
3fiber	
	251					300
8fiber	..LTNN.KLG	IALDAPFDVI	D..NKLTLLA	GHGLSII.TK	ETSTLPGLVN	
9fiber	..LTNN.KLG	IALDAPFDVI	D..NKLTLLA	GHGLSII.TK	ETSTLPGLRN	
15fiber	..LTNN.RIG	IALDAPFDVI	G..GKLTLLA	GHGLSII.TE	ETSPLPGLVN	
17fiber	..LANNKLE	LVYVDPFEVS	A..NKLSLKV	GHGLKILDDK	SAGGLKOLIG	
2fiber	PIYVNNKGIG	IKISGPLQVA	QNSDTLTVVT	GPGVTVEQNS	LRTKVAGAIG	
5fiber	PIYTQNGKLG	LKYGAPLHVT	DDLNTLTVAT	GPGVTINNTS	LQTKVTGALG	
4fiberLNTLVSAF	GSGGLSGSA	LAVQLASPLT	
40-1fiber	PIAVSANSIT	LATAAPLTVS	N..NQLSINT	GRGLVITNNA	VAVNPTGALG	
41fiber	PIAVSANSIT	LATAAPLTVS	N..NQLSINA	GRGLVITNNA	LTVNPTGALG	
40-2fiber	PFTVSGGNLN	LATSAPLSVQ	N..NSLSLGV	NPPFLITDSG	LAMDLDGDLA	
12fiber	PLNSTGSTLS	LSVANPLTIS	Q..DTLTVST	GNGLOVSGSQ	LVTRIGDGLT	
3fiber	...TTDGSLE	ENIKVNTPLT	KSNHSINLPI	GNGLOIEQNK	LCS.....	
	301					350
8fiber	
9fiber	
15fiber	
17fiber	
2fiber	YDSSNNMEIK	TGGGMRIIN..	NNLLILDVDY	PFDAQTKLRL	KLGGPLYIN	

FIGURE 8B

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5fiber	FDSQGNMQLN	JA	LRIDSQ	NRRLLILDVSY	PFDAQNQL	RLGQGPLFIN
4fiber	FDDKG
40-1fiber	FNNTGALQLN	AAGGMRVDGA	N..LILHVAY	PFEAINQLTL	R.....
41fiber	FNNTGALQLN	AAGGMRVDGA	N..LILHVAY	PFEAINQLTL	R.....
40-2fiber	LGG.SKLIIN	LGPGLQMSNG	A..ITL....	ALDAALPL..Q
12fiber	FDN.GVMKVN	VAGGMRTSGG	R..IILDVNY	PFDASNLSL	RRGLGLIYNQ
3fiber
	351					400
8fiber	TLVVLTGKGI
9fiber	TLVVLTGKGI
15fiber	TLVVLTGKGL
17fiber	TLVVLTGKGI
2fiber	ASHNLDINYN	RGLYLFNASN	NTKKLEVSIX	KSSGLNFDNT	AIAINAGKGL
5fiber	SAHNLDINYN	KGLYLFNASN	NSKKLEVNLS	TAKGLMFDAT	AIAINAGDGL
4fiber	...NIKITLN	RGLHVTTGDAIESNIS	WAKGIRFEDG	AIATNIGKGS
40-1fiber
41fiber
40-2fiber	YKNNQLQLRIGS
12fiber	STNWNLTTDIST
3fiber
	401					450
8fiber	GTDLNNNGG	...NICVRVG	E.....GGGLS	FNDNGDLVAF
9fiber	GTESTDNGG	...TVCVRVG	E.....GGGLS	FNNDGDLVAF
15fiber	GTDTTDNGG	...SIRVRVG	E.....GGGLS	FNEAGDLVAF
17fiber	GTENLQNTDG	SSRGIGISVR	A.....REGLT	FDNDGYLVAV
2fiber	EFDNTSESP	DINPIKTKIG	SGIDYNENGA	MITKLGAELS	FDNSGAITIG
5fiber	EFG..SPNAP	NTNPLKTKIG	HGLEFDSNKA	MVPKLGTLGS	FDSTGAITVG
4fiber	RFGTSSTETG	VNNAYPIQVKLGSGLS	FDSTGAIMAG
40-1fiberLE	NGLEVTNGGK	LNVKLGSGLQ	FDNNGRITIS
41fiberLE	NGLEVTSGGK	LNVKLGSGLQ	FDSNGRIALS
40-2fiber	ASALIMSGVT	QTLNVNANTS	KGLAIENNS	LNVKLGNGLR	FDSWGSIAVS
12fiber	EKGLMFSGN	...QIALNAG	OGLTFNNGQ	LRVKLGAGLI	FDSMNIALG
3fiberKLGNGLT	FDSNSIALK
	451					500
8fiber	NKKEDK	...RTLWTTTDP	SPNCRID	...QDKDSKLSLV	LTKCGSQILA
9fiber	NKKEDK	...RTLWTTTDP	SPNCKID	...QDKDSKLTIV	LTKCGSQILA
15fiber	NKKEDM	...RTLWTTTDP	SPNCKII	...EDKDSKLTIV	LTKCGSQILG
17fiber	NPKYDT	...RTLWTTTDP	SPNCRID	...KEKDSKLTIV	LTKCGSQILA
2fiber	NKNDDK	...LTLWTTTDP	SPNCRIH	...SDNDCKFTLV	LTKCGSQVLA
5fiber	NKNNDK	...LTLWTTTAP	SPNCRLN	...AEKDAKLTIV	LTKCGSQILA
4fiber	NKDYDK	...LTLWTTTDP	SPNCQIL	...AENDAKLTLC	LTMCDSQILA
40-1fiber	NRIQTRSVTS	LTTIWSIS.P	TPNCSIY	...ETQDANLFLC	LTKNGAHVLG
41fiber	NSNRTRSVPS	LTTIWSIS.P	TPNCSIY	...ETQDANLFLC	LTKNGAHVLG
40-2fiber	PTTTT...P	...TTLWTTADP	SPNATFY	...ESLDAKVWLV	LVKCNGMVNG
12fiber	SSSNTFYDP	...LTLWTTTDP	PPNCSLI	...QELDAKLTLC	LTKNGSTVNG
3fiber	NN.....	...TLWTGPKP	EANCIIEYCK	QNPDSKLTIV	LVKNGGIUNG
	501					550
8fiber	NVSLIVVAGR	YKIINNNTNP	...ALKGFTIK	LLFDKNGVLM	ESSN.....
9fiber	NVSLIVVDGK	YKIINNNTNP	...ALKGFTIK	LLFDKNGVLM	ESSN.....
15fiber	SVSLIVVKGK	FSNINNNTNP	NEADKQITVK	LLFDKNGVLM	QGST.....
17fiber	NVSLIVVSGK	YQYIDHATNP	...TLKSFKIK	LLFDKNGVLM	PSSN.....
2fiber	TVAALAV.S	...GDLSSM	TGTVASVSIF	LRFDKNGVLM	ENSS.....
5fiber	TVSVLAV.K	...GSLAPI	SGTVQSAHLI	IRFDKNGVLM	NNSF.....

FIGURE 8C

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4fiber	TVSVLVVRS.	..	GNLNPI	TGTVSSAQVF	LRFDANGV	TEHS.....
40-1fiber	TITIKGLKGA	LREMDNA..	LSVK	LPFDNQGNLL	NCA.....
41fiber	TITIKGLKGA	LREMDNA..	LSLK	LPFDNQGNLL	NCA.....
40-2fiber	TISIKAQKGT	LL..KPTASF	ISFV	MYFYSDGTWR	KNYPVFDNEG
12fiber	IVSLVGKGN	LLNIQSTTTT	VGVB	LVFDEQGRLL	TSTP.....T
3fiber	VVTLMGASDY	VNTLFKNKNV	SINVE	LYFDATGHIL	PDSSSLKTDL
551						
8fiber	..LGKSYWNF	RNQNSIMSTA	YEKAIGFMPN	LVAYPKPTTG	SKKY...ARD	600
9fiber	..LGKSYWNF	RNENSIMSTA	YEKAIGFMPN	LVAYPKPTAG	SKKY...ARD	
15fiber	..MDSSYWNY	RSDNSNLSQP	YKKAUGFMPN	KTAYPKQTKP	TNKEISQAKN	
17fiber	..LDSTYWNF	RSDNLTVSEA	YKNAVEFMPN	LVAYPKPTTG	SKKY...ARD	
2fiber	..LKKHYWNF	RNGNSTMANP	YTNAVGFMPN	LLAYPKTQSQ	T.....AKN	
5fiber	..LDPEYWNF	RNGDLTEGTA	YTNAVGFMPN	LSAYPKSHGK	T.....AKS	
4fiber	..TSKKYWG	KQGDSIDGTP	YTNAVGFMPN	STAYPKTQSS	T.....TKN	
40-1fiber	..LESSTWRY	QETNAVA...	..SNALTFMPN	STVYPRNKTA	D.....PGN	
41fiber	..LESSTWRY	QETNAVA...	..SNALTFMPN	STVYPRNKTA	H.....PGN	
40-2fiber	ILANSATWGY	RQGQSANTN.	VSNAVEFMPN	SKRYPNEKGS	E.....VQN	
12fiber	ALVPQASWGY	RQGQSVSTNT	VTNGLGFMPN	VSAYPRPNAS	E.....AKS	
3fiber	ELKYKQTADFSARGFMPN	TTAYPFVLPN	AGTH...NEN	
601						
8fiber	IVYGNILGG	KPHQ..PVTI	KTTFNQETG.	CEYS	ITFDFSWAKT
9fiber	IVYGNILGG	KPDQ..PVTI	KTTFNQETG.	CEYS	ITFDFSWAKT
15fiber	KIVSNVYLG	KIDQ..PCVI	IISFNED.	SDYS	IVFYFKWYKT
17fiber	IVYGNILGG	LAYQ..PVVI	KVTFNEED.	SAYS	ITFEFVWNKE
2fiber	NIVSQVYLHG	DRTK..PMIL	TITLNGTSES	TETSEVSTYS	MSFTWSWESG	
5fiber	NIVSQVYLHG	DRTK..PVTI	TITLNGTQET	GDIT..PSAYS	MSFSWDWSGH	
4fiber	NIVGQVYMNG	DVSK..PMLL	TITLNGTDDT	T.....SAYS	MSFSYTWING	
40-1fiber	MLI.....	QISP..NITF	SVVYNEINS.	GYA	FTFKW.SAEP
41fiber	MLI.....	QISP..NITF	SVVYNEINS.	GYA	FTFKW.SAEP
40-2fiber	MALTYTFLQG	DPNM..AISF	QSIYN..HA.	IEGYS	LKFTW.RVRN
12fiber	QMVSLTYLQG	DTSK..PITM	KVAFNGITS.	LNGYS	LTFMW.SGLS
3fiber	YIFGQCYKA	SDGALFPLEV	TVMLNKRLPD	SRTSYVMTFL	WSLWAGLAPE	
651						
8fiber	.YVNVEFETT	SFTFSYIAQE	..			672
9fiber	.YVNVEFETT	SFTFSYIAQE	..			
15fiber	.YENVQFDSS	SFNFSYIAQE	..			
17fiber	.YARVEFETT	SFTFSYIAQQ	..			
2fiber	KYTTETFATN	SYTFSYIAQE	..			
5fiber	NYINEIFATS	SYTFSYIAQE	..			
4fiber	SYIGATFGAN	SYTFSYIAQQ	..			
40-1fiber	...GKPFHPP	TAVFCYITEQ	..			
41fiber	...GKPFHPP	TAVFCYITEQ	..			
40-2fiber	...NERFDIP	CCSFSYVTEQ	..			
12fiber	NYINQPFSTP	SCSFSYITQE	..			
3fiber	T.TQATLITS	PFTFSYIRED	D.			

FIGURE 8D

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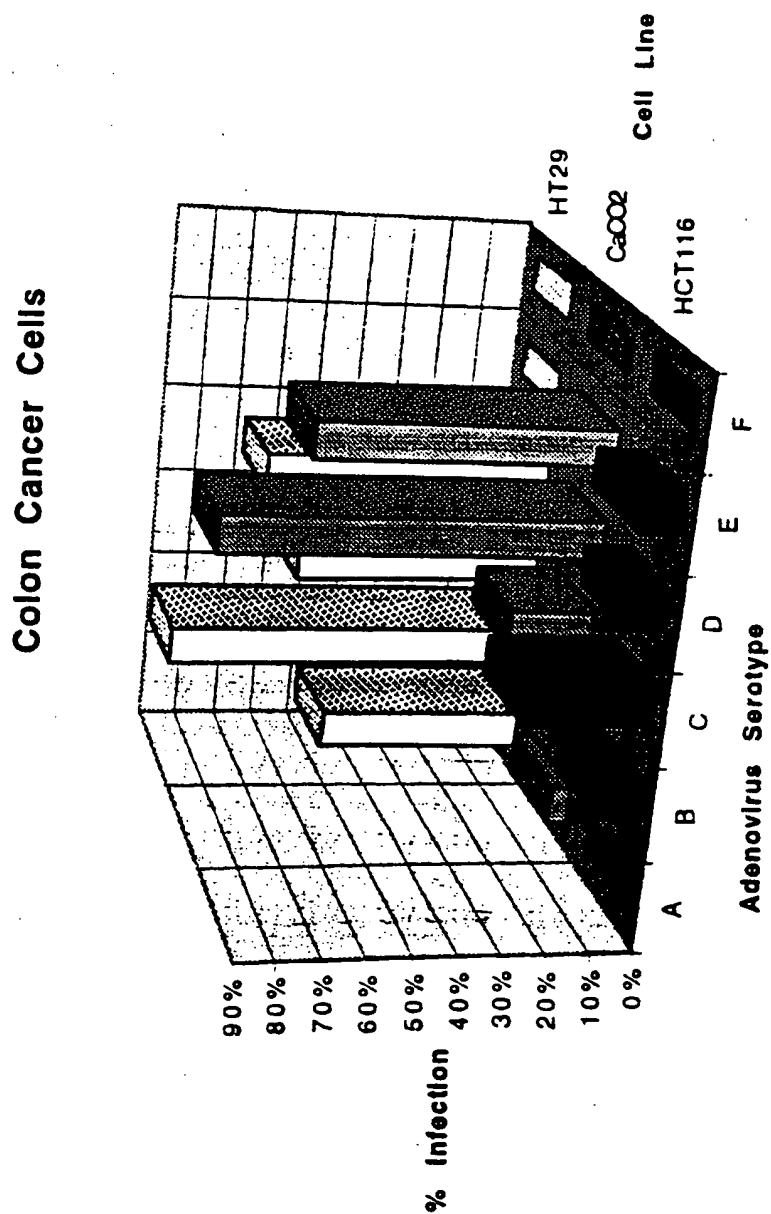
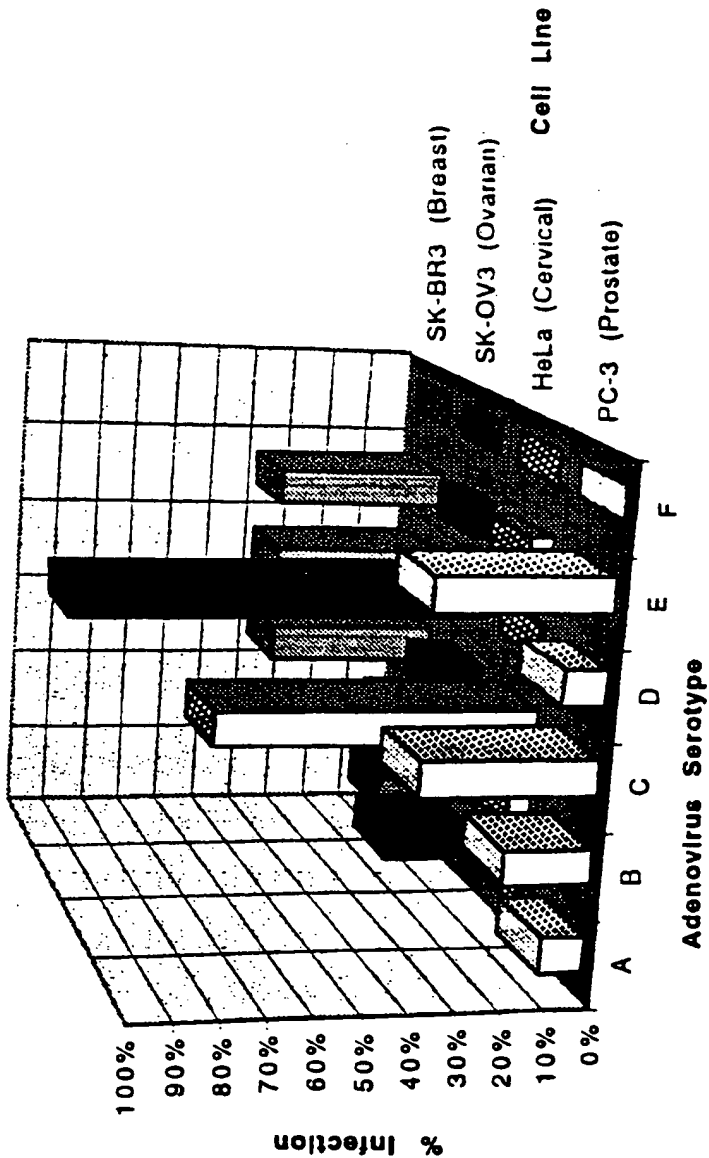


FIGURE 9

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Cancer Cell Lines



EXAMPLE 10

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/21494

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	P.W. ROELVINK ET AL.: "Comparative analysis of adenovirus fiber-cell interaction: Ad2 and Ad9 utilize the same cellular fiber receptor but use different binding strategies for attachment" JOURNAL OF VIROLOGY, vol. 70, no. 11, November 1996, AMERICAN SOCIETY FOR MICROBIOLOGY US, pages 7614-7621, XP002062100 see page 7620, last paragraph	1-13
A	WO 96 26281 A (GENVEC INC ; CORNELL RES FOUNDATION INC (US)) 29 August 1996 see example 7 --- -/--	1,4,6-8, 10,11

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "S" document member of the same patent family

Date of the actual completion of the international search

14 April 1998

Date of mailing of the international search report

123.04.98

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Authorized officer

Cupido, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/21494

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		Relevant to claim No.
Category *	Citation of document, with indication, where appropriate, of the relevant passages	
A	J. GALL ET AL: "Adenovirus type 5 and 7 capsid chimera: Fiber replacement alters receptor tropism without affecting primary immune neutralization epitopes" JOURNAL OF VIROLOGY., vol. 70, no. 4, April 1996, pages 2116-2123, XP002050655 see the whole document ---	1,4,6-8, 10,11
P,X	WO 97 12986 A (CORNELL RES FOUNDATION INC) 10 April 1997 see page 15, line 1 - line 7 -----	1,2,13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/ 21494

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 11 to 13
because they relate to subject matter not required to be searched by this Authority, namely:
Although these claims are directed to a method of treatment of the human or animal body, the search has been carried out and based on the alleged effects of the adenoviral vector
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.